ENDOCRINOLOGY

VOLUME 43 AUGUST, 1948 NUMBER 2

COMB-TESTIS RELATIONSHIP

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IT HAS been known for some time that when cockerels are caponized, the comb regresses. Also when an androgenic substance is applied topically to the comb of a capon, or when injected intramuscularly, the growth of the comb is resumed. This has been made the basis of a quantitative measure of the androgenic activity of crystalline androgens, and tissue and urine extracts. Later, it was shown by Buchner *et al.* (1934), Hoskins and Koch (1939), Marlow and Payne (1940) and Zowadowsky (1935) that the removal of the comb results in significant increase in the size of the testis of a cockerel.

There would then appear to be a reciprocal relationship between the comb and testis. Whether or not this reciprocity also includes the pituitary has not been established. Zowadowsky (1936) upon feeding ground cockerel's combs and injection of impure alcoholic extracts in male rats reports a smaller testis, seminal vesicles and prostate glands. Hoskins and Koch (1939) were not able to confirm Zowadowsky's results but on the contrary noted a slight but not a mathematically significant increase in the size of above-mentioned organs. They further tried the same experiment on fowls which perhaps should be more receptive to dried ground cockerels' combs in the diet than rats and again noted slight but not significant increase in body weight, testis weight to body weight ratio, and in comb growth. Feeding of dried comb tissue to capons failed to show an active substance. No inhibitory substance could be found.

Landauer (1942) in a series of experiments obtained testis growth by several methods: Removal of wattles, comb and wattles, and the posterior half of the comb gave increased testicular weights, but doubtful or negative results were obtained when the combs alone were removed, edges of comb or wattles clipped, or horizontal slits made in the comb. Zowadowsky explained his results on a reciprocal basis which may be expressed as below.

Received for publication April 20, 1948.

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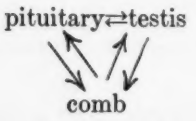
pituitary⇄testis→comb

Koch went one step further and stated that the interrelationships between the three organs were perhaps better expressed by:



but suggested that further work must be done to show the involvement of the pituitary. Landauer's (1942) explanation is that there is no endocrine interrelationship but that the results noted were caused by trauma.

In an effort to aid in clarification of this problem, the author outlined an experiment removing the comb from baby chicks 3 days old in which trauma certainly would be little or negligible. The combs were removed by scissors with little or no loss of blood. It is believed that this treatment to young chicks would reduce traumatic shock to a minimum or eliminate its effect. No work has been reported on baby chicks in this regard as most birds were used at 21 days or over when cutting the combs produces quite a traumatic shock, although it is difficult to see how the effects could continue over a peroid of several months. Purified comb extract was used on dubbed birds and on normal cockerels, and its effect studied on the growth of the testis, comb, wattle and body. Young and old, male and female rats were also included. Observations on the vaginal smear of the older female rats were made and vaginal introitus of the young female rats studied. Weight of seminal vesicles and testis of both old and young male rats were obtained.

EXPERIMENTAL

Preparation of Experimental Animals

The combs of single comb, white leghorn cockerels three days old were removed by applying a pair of scissors to the posterior portion of the comb and snipping the comb off to the beak close to the head. Very little bleeding, and in many cases none, was noted. If left alone a rosette of comb material grows back slowly. To minimize this a saturated solution of trichloroacetic acid was placed on the wound and renewed growth was minimized, but not prevented with the one application.

Preparation of Purified Extract

The combs from single comb white leghorn cockerels of broiling age which were removed soon after death, were ground with a meat chopper and extracted with 95 per cent alcohol in a continuous extractor (1935). This extract was chilled overnight to remove neutral fats. The alcoholic extract was evaporated to dryness by distilling under reduced pressure. Absolute

alcohol was added in several portions and removed under suction, thus removing final traces of water. This was then dissolved in the least quantity of absolute alcohol possible and chilled to remove toxic sterols, and remaining neutral fats. The alcoholic solution was again evaporated to dryness as above. The residue was dissolved in ether and 4 volumes of acetone added to remove phospholipins. The ether-acetone solution was then evaporated by distillation and a calculated amount of corn oil (Mazola) added, just before all of the solvent was removed. Another portion of the ground comb tissue was extracted thoroughly with 0.9 per cent saline. This saline solution was adjusted to a pH of maximum flocculation of protein (pH 4.14). This protein residue was centrifuged out, taken up in 0.9 per cent saline and adjusted to pH of 7.33.

Injection of the purified alcoholic extract in oil or the neutral saline emulsion in rats showed none of the toxic effects reported by Zowadowsky (1935–36) which produced, according to reports, decreased growth, decreased size of testis, etc. Daily injections were carried out over periods of 10 days or more without toxic symptoms.

Histological examination of the testis, combs, uteri and ovaries of birds treated with comb extracts show no degenerative effects when compared to saline injected controls.

RESULTS OF ADMINISTRATION OF SALINE EXTRACT

A dosage equivalent to $3\frac{1}{3}$ gm. of comb tissue was injected daily for 10 days in old and young normal female and in young male rats. No disturbance of the estrus cycle of the females could be detected, and no effect of vaginal introitus of young females were noted. Ovarian weight/body weight and weight of uterus to body weight ratios of the females showed no calculated significant differences over controls. Weight of seminal vesicle to body weight and weight of testis to body weight changes were not significantly different.

ADMINISTRATION OF PURIFIED ALCOHOLIC EXTRACT

A dosage equivalent to 10 gms. of comb tissue was injected into young and old female and young male rats with similar negative results as cited above (Table 1). It is expected that the genitalia of cocks would more likely be affected by comb tissue than those of rats as suggested by Hoskins and Koch (1939). The purified alcoholic extract dissolved in corn oil (Mazola) was spread on the head furnishing of cockerels by use of a 1 cc. hypodermic needle following generally the procedure of Frank *et al.* (1942).

Four pens of fowls were prepared:

1. Pen #1 contained 25 baby chicks from which the comb had been removed and alcoholic extract of comb in Mazola applied daily. (Dosage equivalent to 0.69 gm. fresh comb tissue daily per fowl was administered.)

2. Pen #2 containing 25 baby chicks from which the combs had not been removed and alcoholic extract of comb tissue in corn oil

(Mazola) applied daily. (Dosage equivalent to 1.15 gm. fresh comb was used daily.)

3. Pen #3 containing 25 baby chicks from which the combs had been removed but corn oil (Mazola) administered daily.

4. Pen #4 containing 25 chicks in which the combs had not been removed but corn oil (Mazola) added daily.

The combs were removed from chicks in pens 1 and 3 at 3 days of age, and the alcoholic extracts applied to the comb or rosette for a period of 38 days twice daily. The feathers had been removed by scissors in the vicinity of the comb so as to minimize loss of material

TABLE 1. EFFECT OF EXTRACTS OF COMB TISSUE ON RAT GENITALIA

Treatment	Body weight (av.)	Females (Response)			
		Uterine weight (av.)	Ovarian weight (av.)	Uterus (mg.) Body weight (gm.)	Ovaries (mg.) Body weight (gm.)
Oil	62	62	26	1.00	.42
Comb extract	57	52	23	.91	.40
Saline	49	47	27	.96	.56
Saline extract	43	32	17	.74	.40
		Males (Response)			
		Testis weight	Seminal vescl. weight	Testis (mg.) Body weight (gm.)	Sem. vescl. (mg.) Body weight (gm.)
Oil	49	563	14	11.50	.29
Comb extract	56	611	16	10.90	.29
Saline	56	590	13	10.60	.21
Saline extract	49	588	15	12.00	.30

on the feathers. The total amount of alcoholic extract or oil administered to the combs of the fowls in a pen for a given number of fowls was recorded for the entire period rather than an individual record of each chick. At the end of this time the chicks were fasted for 36 hours to empty the gut, and then killed. Combs, wattles and testis were removed and weighed. The average result of these weights is recorded in Table 2.

It is observed that on the basis of average figures that the testicular weight of fowls receiving the extract on a normal comb and on the fowls with comb removed, and receiving corn oil (Mazola) only, were identical, while the one receiving the extract on the rosette was greater than either. This latter suggests an additive effect of removal of comb and application of the extract. It is not strictly proportional due first perhaps to less surface for absorption of the extract on the normal comb rather than on the rosette and secondly in this experiment to a difference in daily dosage (0.69 gm. equivalent of fresh tissue for Pen #1 and 1.15 gm. equivalent of fresh tissue for Pen #3). Calculations on this basis indicate that the effect of removing the comb alone on the testis to body weight ratio would be .08, while the

TABLE 2. EFFECT OF PURIFIED COMB EXTRACT ON CHICK COMB, WATTLES AND TESTIS

Pen No.	No. animals	Initial body weight	Body weight (av.)	Comb weight (av.)	Wattle weight (av.)	Testis weight (av.)	Ratios to body weight		
							Comb (av.)	Wattle (av.)	Testis (av.)
1	24	43.5 g	316	.399 g*	.392 g	.160 g	1.22 †	1.25 ± .13	.51 ± .06
2	26	41.1 g	324	3.504 g	.456 g	.139 g	10.56 ± .01	1.36 ± .19	.43 ± .04
3	21	45.0 g	310	.430 g	.286 g	.126 g	1.30 *	.90 ± .10	.43 ± .04
4	24	41.5 g	299	2.072 g*	.327 g	.085 g	6.89 ± .01	1.08 ± .01	.28 ± .02

* Rosette is a growth of comb after removal.
† Cannot be computed as combs were removed and some were treated with concentrated trichloroacetic acid and growth was hence irregular.

extract gives an added .15 *increase* over the normal effect. If both pens had received the larger quantity (1.15 gm. daily) the average testicular weight could be calculated at .59 instead of .51 maintaining a significant difference. Table 3 shows the calculated "t" values for this experiment.

The fowls in pens 2 and 4 from which the comb has not been removed are the only ones for which a "t" value for comb differences could be evaluated. Using Fisher's (1938) tables of "t" values the increase in size of the combs of the chicks in pen #2 receiving the comb extract over the ones in pen #4 receiving oil only is highly significant. The increase in wattle growth was not as significant as the comb growth. The ratio of organ weight in milligrams over the body weight in grams was used throughout in order to equalize the size relationships. Body weights of the fowls treated with the comb extract or the controls receiving oil alone does not show a significant difference.

ASSAY OF ANDROGENIC SUBSTANCE

Twenty-five 2 day old single comb white leghorn cockerels were treated with the alcoholic extract of comb as indicated in an earlier

TABLE 3. COMPARATIVE "T" VALUES OF COMB, WATTLES AND TESTIS OF CHICKS

Pens	Ratio to body weights			Testicular weig ts	
	Combs "t" value	Wattles "t" value	Testis "t" value	"t" value	"t" significant value
1&2	*	.7015	.1013	.7271	2.660
1&3	*	2.116	.1064	1.411	2.660
1&4	*	1.095	3.803	3.911	2.660
2&3	*	2.273	†	.6001	2.660
2&4	2.645	1.854	2.942	3.193	2.660
3&4	*	.1143	3.372	2.422	2.660

* Could not be compared as comb had been removed from birds in pens 1 or 3.
† Could not be calculated as the difference of their means is zero.

paragraph. Fifteen were treated with a known quantity of androsterone acetate. Ten received corn oil (Mazola). This was applied to the comb with a 1 cc. Luer syringe. To minimize the spreading of the oil or extract to the feathers, a commercial hair remover was applied around the comb and on top of the head to remove downy feathers. After standing some ten minutes, the hair remover was washed off carrying with it all the fine hair or feathers on the head of the chick. This allowed a clean head with little danger of the oil spreading to any feathers.

To prevent too wide spreading of the vehicle over the head near the comb a divided daily dosage was applied twice daily. A maximum average dosage of .01 to 0.2 ml. was applied, as any larger volume

TABLE 4. ASSAY OF ANDROGENS

Pen No.	No. animals	Body weight		Comb weight	Comb Size		Testis weight	Ratio (mg.)/Body Wt. (gm.) Dcmg.		
					L	H				
		Int.	End	(av.)	(av.)	(av.)		Comb	Testis	
		gm.	gm.	mg.			mg.			
1*	23	40	64	34	10.8	3.6	18	.52	.26	11.8
2†	15	40	62	79	11.8	4.4	15	1.27	.26	37.0
3‡	10	40	65	29	10.3	3.5	19	.44	.29	8.5§

* 1 = on Extract of comb; † 2 = Androsterone-acetate; ‡3 = Mazola.

§ A personal communication from one of the authors (E.K.) whose method for assay of androgens by the chick method (8) implies that in their hands #3 above would have a dcmg. of zero.

(.05 ml.) spread too much over the head. This was continued for seven days and on the 8th day the chicks were sacrificed. The comb and testis were removed and weighed, and the comb measured. The differences in the weights of the wattles were too small to be significant. Each experimental chick received an average total dosage for the seven days equivalent to 11.5 gm. of fresh comb tissue. The control chicks received an average of 13.5 micrograms of androsterone acetate over the same period. It is very evident from Table 4 that the body weight changes were insignificant. It is clear that the comb had begun to increase, with only a slight trend in decreased weight of the testis noted. Seven days may be too short a time with this dosage for critical evaluation.

Calculations according to the method of Frank and others (1942) indicates that the comb extract contained 11.8 dcmg. (chick) as compared to 37.0 cdmg. of the androsterone acetate. This would seem to indicate that both the short time and the concentration of the extract was too little to give mathematically significant figures.

DISCUSSION

This increase in testicular weight when the comb is removed parallels closely the results of all other investigations except that of

Landauer (1942) who gets significant increase in testicular weight only if both comb and wattles are removed, and not when the comb alone is removed.

Contrary to the report of Zowadowsky (1935), but in slight agreement with Hoskins and Koch (1939), injection or topical application of an oil containing an alcoholic extract to the comb, increases the size of the comb and testis. None of the fowls were fed dried comb tissue, but the more concentrated non-toxic extract was used by topical application.

An androgenic assay conducted according to the method of Frank *et al.* (1942) using 3 day old baby chicks was made and an increase in the size of comb over that of control was noted. Androsterone acetate was applied in oil as a reference substance.

CONCLUSIONS

Removal of the combs of chicks 3 days of age results in a significant increase in the size of the testis, but not a significant increase in the size of wattles.

Topical application to the comb of a chick of a purified alcoholic extract of rooster combs resulted in an enlargement of the testes and combs over that of controls.

Quantitatively assay of the alcoholic extract indicates the presence of an androgenic compound of unknown composition, which may be effective through the pituitary, but it is not present in sufficient quantity in the dosage applied to alter the action of the pituitary. Berdnikoff and Chauncy (1935) obtained some androgenic activity upon hydrolysis of combs with hot concentrated trichloroacetic acid.

Using baby chicks as subjects where little trauma is encountered in dubbing, it is believed that there is some effective androgenic substance in the comb of roosters. Landauer (1942) did not get significant results with the removal of comb, clipping the comb, or slitting the comb, although the removal of both combs and wattles, wattles alone, or removal of posterior half of the comb was effective. Trauma would be encountered in all events.

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THE EFFECT OF INANITION ON THE INACTIVATION OF ESTROGEN BY THE LIVER[1]

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IT HAS been established that exogenous estrogens can be inactivated by the animal organism with great facility. Zondek and Sklow (1941) have shown that only 5 per cent of the estrogen administered to rats can be recovered from the animal body three hours after its subcutaneous injection. Heller (1940); Singher *et al.* (1944) have demonstrated by in vitro studies that the liver is the organ involved in this degradation mechanism. Talbot (1939), Schiller and Pincus (1944) and others have shown an impairment of inactivation in the presence of hepatic parenchymal damage in the rat and Glass, Edmondson and Soll (1940) have demonstrated the same phenomenon in patients with advanced hepatic cirrhosis.

Golden and Sevringhaus (1938) implanted ovaries into a site drained by the portal vein and discovered that the rats showed no sign of estrus as determined by vaginal smear. However, if these ovaries were re-implanted into the axilla, estrus re-appeared. Biskind and Marks (1939) demonstrated a similar phenomenon with pellets of estrone implanted into the spleen. Biskind and Biskind (1942) carried out further studies to show that when rats were placed on a diet deficient in components of the vitamin B complex the liver lost its ability to inactivate the estrogen. These studies were confirmed and it was demonstrated that the important components of the B complex were thiamine and riboflavin (Segaloff and Segaloff, 1944). In the absence of either dietary thiamine or riboflavin the degradation mechanism of the liver for estrogens was impaired.

Drill and Pfeiffer (1946) have presented data to question the role of vitamin B in the inactivation mechanism of estrogens in rats, and showed that inanition is a much more important factor. Segaloff, however, previously had attempted to rule out the factor of inanition. It appeared profitable to reinvestigate this problem and attempt to separate the influence of the two factors, namely, vitamin B deficiency and inanition.

Received for publication June 18, 1948.

[1] Aided by a grant recommended by the Committee on Growth, National Research Council to the American Cancer Society, administered by Dr. E. T. Engle.

METHODS AND MATERIALS

Adult female rats of the Long-Evans strain weighing approximately 200 Gm. were ovariectomized; 14 days later pellets of α-estradiol (1–3 mg.) were implanted under the capsule of the spleen. After a period of 14 days when the vaginal smears were of the diestrus type, the animals were placed on a vitamin B deficient diet (Drill and Pfeiffer, 1946) and allowed to eat ad libitum. The daily consumption of food was recorded.

Two groups of three rats were housed in each metabolism cage. Thiamine determinations were performed by a modification of the thiochrome method (Lowry, Bessey and Davis, 1948) on 48 hour specimens of urine collected in Erlenmeyer flasks containing 0.5 ml. glacial acetic acid. The animals were weighed at various intervals and vaginal smears were taken daily. The same six animals were studied through: 1) a period of vitamin B deficiency; 2) a period of restricted food intake with adequate vitamin B; 3) a period of unrestricted food intake and sufficient vitamin B.

At the conclusion of the experiment the animals were sacrificed, the pellets were removed from the spleen, cleaned, dried and weighed. The amount of estradiol absorbed averaged 12 micrograms/day/rat.

RESULTS

During the 31 days in which the animals were on a diet deficient in the components of the vitamin B complex (Figure 1, A) they lost an average of 28.2 per cent of their initial body weight. The food intake fell from approximately 40 Gm./day/3 rats to 10 Gm. The rats exhibited the physical manifestations of vitamin B deficiency. Thiamine excretion quickly fell to less than 1 microgram/48 hours/3 rats, which is an indication of decreasing thiamine stores (Holt, 1943). After approximately 18 days on this diet, the vaginal smears were continually of an estrus type.

The period of inanition with sufficient vitamin B consisted of 8 days (Figure 1, B) in length during which time the animals were fed 10 Gm./day/3 rats (an average of the previous week's daily food intake). In addition they were injected with 0.05 ml. Solu B.[2] Each animal continued to lose an average of 4 Gm. of body weight for the 8 day period. They appeared to be in better physical condition, however, and were much more active and quite vicious. The urinary excretion of thiamine rose to levels as high as 50 micrograms for a 48 hour period. However, the vaginal smears continued to be of the estrus type.

After this 8 day period of restricted food intake the animals were allowed to feed adlibitum (Figure 1, C) the same diet to which the components of the vitamin B complex had been added (Drill and Pfeiffer, 1946). The food intake quadrupled overnight; however, the thiamine excretion decreased due to diminished thiamine intake. The animals regained approximately 50 per cent of the weight lost during

[2] Each 0.05 ml. Solu B contains 50γ thiamine, 50γ riboflavin, 25γ pyridoxine, 250γ calcium pantothenate and 1.25 mg. nicotinamide.

the thiamine deficient period. Within 5 days after the institution of this diet, the vaginal smear began to revert to the diestrus type. A typical experiment is represented in Figure 1. The data for the other animals are practically identical with the one illustrated.

DISCUSSION

From the data presented, it appears possible to separate the effects of vitamin B deficiency and the concomitant effects of inanition. In

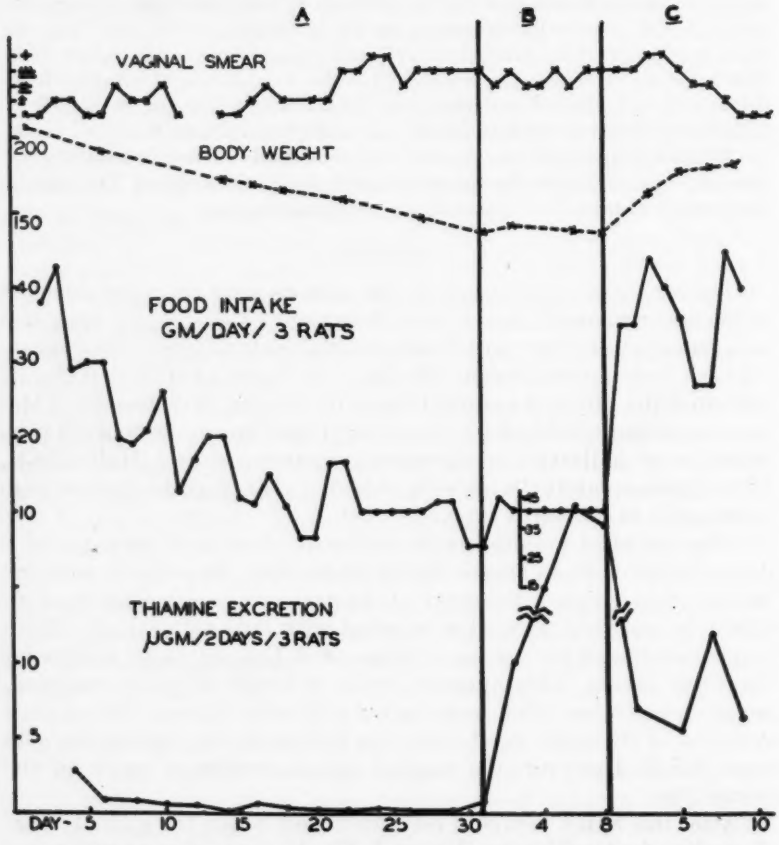

FIG. 1. A = Period of vitamin B deficiency; B = Period of restricted food intake plus vitamin B administration; C = Period of unrestricted food intake and sufficient vitamin B. The vaginal smears and body weights are of one representative animal of the group. The food intake and thiamine excretion are the total of three. The classification of vaginal smears is that employed by Kahnt and Doisy (1928):

\+ Cornified smear
± ± ± Cornified + epithelial cells
± ± Epithelial cells
± Leucocytes + epithelial cells

spite of massive quantities of vitamin B, the rats were unable to inactivate estradiol when the diet was restricted. However, on the resumption of ad libitum feeding during which time the rats began to regain part of the weight lost, estrogen inactivation appeared.

Biskind *et al.* and Segaloff claim that it is the vitamin deficiency which interferes with the degradation mechanism, although their experiments were not well controlled for caloric intake. György (1945) has shown that hepatic damage caused by a low protein diet will result in the same phenomenon.

It is entirely possible that several factors are involved to cause the liver to lose its ability to inactivate estrogens. Protein deficiency and inanition have been shown by György, by Drill and Pfeiffer and in this study to be one of these factors. In the work of Biskind *et al.* and Segaloff, it should be noted that on the vitamin deficient diet the vaginal smears changed to the estrous type after only a few days (4–8), while in this experiment approximately 21 days were necessary to reach the estrous state. This dissimilarity may be due to differences in the strain of rats used. It is improbable, however, that it is due to differences in the synthesis of thiamine and riboflavin because these rats could not degrade estrogens when on restricted caloric intake even though the diet was supplemented with large amounts of vitamin B complex.

SUMMARY

Spayed female rats with pellets of α-estradiol implanted in the spleen and on a vitamin B deficient diet show no signs of estrus, as evidenced by vaginal smear, until they have lost approximately 20 per cent of the body weight. In spite of the administration of large amounts of thiamine and riboflavin, the vaginal smears remain of the estrous type, when the food intake is restricted. However, when the rats are then allowed to eat ad libitum, the liver regains its ability to inactivate estrogen. It appears that it is not deficiency of the B vitamins but the concomitant inanition which impairs the degradation mechanism for estrogens in the rat liver.

ACKNOWLEDGMENTS

I wish to express my appreciation to Mrs. Ruth S. Finkel for her technical assistance and to Dr. Edward Henderson of Schering Corporation for supplying the α-estradiol.

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EXCRETION OF 17-KETOSTEROIDS BY MALE RABBITS DURING ALTERED GONADAL FUNCTION

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BY STANDARDIZATION of environmental conditions and selection of animals for such variables as health, weight, age, nutrition, it is possible to control factors which have been indicated as influencing the excretion of 17-ketosteroids (Landau et al., 1948; Forbes et al., 1947). Proper use of such controls should provide opportunity for critical investigations of 17-ketosteriod metabolism.

Relatively few studies have reported the urinary 17-ketosteriod excretion in laboratory animals. They have been assayed in the rat (Lampton and Miller, 1941), mouse (Karnofsky et al., 1944), chimpanzee (Dorfman et al., 1947), and rabbit (Kimeldorf, 1948). Unfortunately, values reported by these investigators are not strictly comparable because of differences in assay techniques.

The purpose of the present investigation is to study alterations in 17-ketosteroid excretion experimentally induced in rabbits whose normal excretion level has been established.

MATERIALS AND METHODS

Data reported in these investigations were derived from the study of 20 New Zealand Albino male rabbits. All were sexually mature and weighed 9.5–12.5 pounds with only three animals weighing more than 11.5 pounds. They were fed complete rabbit pellets and received water in heavy stone crocks to avoid urine dilution.

The methods used to collect urines, fractionate the extracts, and assay have been described in detail (Kimeldorf, 1948). Briefly, crude neutral steroid ether extracts of urine were fractionated with Girard's Reagent T by the micro-method of Pincus (1945). Resultant ketonic fractions were assayed by the Talbot et al. (1940) anhydrous-Zimmerman reaction. Assays were read by the aid of an Evelyn photoelectric colorimeter calibrated with androsterone and dehydroisoandrosterone. The quantity of 17-ketosteroids was determined for total 48 hour volumes of urine excreted by individual animals. Each urine was assayed in triplicate and standards were run with nearly all series of assays.

Received for publication June 19, 1948.

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TABLE 1. AVERAGE EXCRETION OF URINARY 17-KETOSTEROIDS (MG./48 HOURS) FOR EACH CONDITION INDICATED

	No. of collections	Total 17-KS	S.E.
Normal	109	2.31	0.10
Castrate	33	1.35	0.10
Cryptorchid	17	1.40	0.07
Cryptorchidectomized	11	1.29	0.29
Pituitary-injected	12	1.38	0.22
Testosterone-implanted	13	3.07	0.27

EXPERIMENTAL PROCEDURES AND RESULTS

Casteration. Ten animals were castrated to determine the total 17-ketosteroid excretion of rabbits in the absence of testicular secretions. The normal excretion of each animal was determined previously so that quantitative changes with castration could be more accurately evaluated. Following castration the animals were allowed three weeks recuperative period. Thirty-three urines of 48 hour duration were collected and assayed.

The mean excretion level of animals in the castrate condition was 58% of the mean excretion level of normal animals. Table 1 summarizes these data. A comparison of the two excretion levels for individual animals is presented in Table 2 and indicates an average decrease of 41% with castration.

Animal B castrate excretion of 17-ketosteroids was determined under different conditions than those of other castrates. The excretion level of this animal was determined seven months after castration instead of the usual three weeks. It is to be noted (Table 2) that this animal had the highest 17-ketosteroid excretion of any castrate.

Experimental cryptorchidism. Three normal rabbits were made cryptorchid by retracting their testes into the abdominal cavity and

TABLE 2. COMPARISON OF THE AVERAGE EXCRETIONS OF TOTAL 17-KETOSTEROIDS AND URINE VOLUMES FOR INDIVIDUAL ANIMALS IN NORMAL AND CASTRATE CONDITIONS

Animal	Normal excretions			Castrate excretion			Per cent decrease
	No. of urines	17-KS mg./48 hrs.	Urine ml./48 hrs.	No. of urines	17-KS mg./48 hrs.	Urine ml./48 hrs.	
B	9	2.27±0.19	402	4	1.79±0.23	378	21.1
H	3	2.56±0.27	852	3	1.64±0.48	846	39.9
I	6	2.35±0.08	318	3	1.45±0.17	338	38.1
J	4	2.50±0.05	459	3	0.96±0.09	418	62.0
K	4	2.76±0.34	467	3	1.52±0.33	536	44.8
L	4	2.01±0.19	270	3	1.37±0.01	225	31.8
M	3	1.88±0.38	422	3	1.32±0.50	356	29.7
O	3	1.91±0.23	283	4	0.82±0.50	392	56.8
P	—	2.31±0.10*	—	4	1.29±0.35	373	44.1
Q	—	2.31±0.10*	—	4	1.28±0.19	307	44.5

* Excretion level of colony.

ligating them to the abdominal wall with minimal disturbance to circulation. As an added precaution the gubernaculum was ligated and cut. A total of seventeen 48-hour urines were assayed. Animals P and Q were identical with the rest of the colony and a normal excretion level was not determined separately for these two animals.

In order to establish a definitive condition of cryptorchidism a period of four weeks elapsed before collection and assay of the urines. The level of 17-ketosteroid excretion was that of castrates (Table 1). To determine subsequent effects of prolonged experimental cryptorchidism a period of eight weeks was allowed to elapse after the previous urine collections and a second series of collections were

TABLE 3. COMPARATIVE EFFECTS OF EXPERIMENTAL CRYPTORCHIDISM AND SUBSEQUENT CRYPTORCHIDECTOMY REFLECTED IN THE AVERAGE TOTAL 17-KETOSTEROIDS EXCRETION (MG./48 HRS.) OF INDIVIDUAL ANIMALS. THE NUMBER OF 48-HOUR COLLECTIONS ASSAYED ARE IN PARENTHESES

Animal	Normal excretion	Cryptorchid excretion	Castrate excretion
M	1.88 ±0.38 (3)	1.28 ±0.12 (6)	1.32 ±0.50 (3)
P	2.31 ±0.10 (—)	1.54 ±0.36 (6)	1.29 ±0.35 (4)
Q	2.31 ±0.10 (—)	1.40 ±0.07 (5)	1.28 ±0.19 (4)

made. The results confirmed the first assay series and established the 17-ketosteroid excretion at the depressed level of castrates.

The cryptorchid rabbits were converted subsequently to castrates by cryptorchidectomy. The gonads were removed and examined microscopically. Sections showed the tubule degeneration characteristic of cryptorchid testes while interstitial cells appeared unaffected. Three weeks after castration eleven 48-hour urine collections were made and assayed. There was no significant change in 17-ketosteroid excretion from the cryptorchid condition (Table 3).

Testosterone pellets. The effectiveness of an androgenic precursor upon the 17-ketosteroid excretion was studied in two castrated animals. Rod-shaped pellets of crystalline testosterone (Oreton F, Schering) weighing 25.0 mg. were used. A single pellet was inserted subcutaneously into the medial left thigh region of each animal. Two days following pellet implantation urines were collected continuously for 14 days. At the end of this period the pellets were removed, dried and weighed. The pellet removed from animal H indicated an absorption of 6.8 mg., while the pellet recovered from rabbit K had decreased 7.2 mg. in 16 days.

Table 4 describes the conditions of the experiment and the 17-ketosteroid excretion for the last 14 of the 16 day period. The values indicate an immediate effective increase of 17-ketosteroid excretion within two days of implantation and than a gradual increase for the next 10 days. The greatest increase during the observed period occurred 15 and 16 days after implantation of the pellets.

TABLE 4. EFFECT OF IMPLANTED TESTOSTERONES PELLETS (25.0 MG.) UPON THE CONTINUOUS 17-KETOSTEROIDS EXCRETION (MG./48 HRS.) OF CASTRATED MALE RABBITS. THE NUMBER OF URINES ASSAYED ARE IN PARENTHESES

Animal:	H	K
Normal excretion:	2.56 ±0.27 (3)	2.76 ±0.34 (4)
Castrate excretion:	1.64 ±0.48 (3)	1.52 ±0.33 (3)
Pellet absorption: (By 16th day)	6.8 mg.	7.2 mg.
Collection period	17-KS excreted	17-KS excreted
Days 1–2	—	—
3–4	2.25	2.63
5–6	1.71	2.75
7–8	2.34	2.41
9–10	3.14	3.05
11–12	3.05	2.48
13–14	3.18	3.64
15–16	5.41	6.00

Injection of pituitary material. The effect of anterior pituitary extracts upon the 17-ketosteriod excretion of low-normal animals was investigated in three rabbits. The pituitary extract used was Polyansyn (Armour) which is a polyvalent extract of pituitary prepared after the method of J. B. Collip. Each animal (R, S and T) received subcutaneously 6 cc. of Polyansyn in divided doses over a six day period. Urine collections were made for 14 days following the first injection. Table 5 lists the excretion of 17-ketosteriods prior treatment compared with excretion during treatment. With the possible exception of animal S there was no significant change in 17-ketosteroid excretion.

While the normal values of these animals were exceptionally low there were no discernible pathologies which would invalidate the assay values obtained from them.

DISCUSSION

Urine volumes did not vary significantly for any experimental condition. An occasional individual rabbit consistently excreted unusual volumes of urine. For example, animal N had an average excretion of 1,080 ml./48 hours, whereas the group average was 357 ml./48 hours. Of all animal groups the normal animals yielded the

TABLE 5. RESULT OF INJECTING LOW-NORMAL ANIMALS WITH PITUITARY EXTRACTS UPON THE TOTAL 17-KETOSTEROIDS EXCRETION (MG./48 HRS.). THE NUMBER OF URINES ASSAYED ARE IN PARENTHESES

Animal:	R	S	T
Excretion prior treatment:	1.78 ±0.05 (4)	1.28 ±0.15 (4)	1.93 ±0.12 (3)
Excretion during treatment:	1.60 ±0.24 (4)	1.60 ±0.22 (4)	1.97 ±0.27 (4)

greatest variation in urine volume of any group and varied between 154 to 1,465 ml./48 hours.

In accordance with the evidence obtained gonadectomy depresses the 17-ketosteriod excretion of male rabbits (Tables 1 and 2). Average values obtained before and after castration indicated the contribution of the testes in rabbits is greater than with man. The replacement value of testerosterone pellets corroborates further the importance of testicular secretions to urinary 17-ketosteroids. Subcutaneous testosterone pellets produced a four-fold increase in 17-ketosteroid excretion within 16 days of implantation. Further increases may have been observed with more prolonged study of the urine excretions. The relatively high excretion occurring seven months after castration of animal B is suggestive of a compensatory mechanism developed with time after castration. Such adrenal compensation for gonadal secretions has been postulated for high levels of the female climateric (Hamblen et al, 1941), and for the increase in 17-ketosteroids excreted by castrated patients with prostatic cancer (Scott and Vermeulen, 1942). The changing proportions of constituent 17-ketosteroids (e.g. more dehydroisoandrosterone) in human castrate urine also suggests adrenal compensation in the absence of the testes (Callow and Callow, 1940).

Considerably more difficult to interpret is the depressed excretion of cryptorchid animals. Evidence obtained in the present study would indicate as early as thirty days following experimental cryptorchidism in rabbits in the 17-ketosteroid excretion is reduced to that of a castrate within experimental error of the technique. Assays of urines collected sixty days after surgery indicate no further decrease in 17-ketosteroid excretion. No further decrease with subsequent cryptorchidectomy adds corroborative evidence of the castrate-like excretion of 17-ketosteroids during experimental cryptorchidism. There is evidence indicating animals made cryptorchid do not continue to secrete the normal level of androgens (Korenchevsky, 1930; Nelson, 1934; Hanes and Hooker, 1937). The depressed 17-ketosteroid excretion suggests that normal 17-ketosteroid level of the cryptorchid rabbit is only transitory at best. An alternative possibility suggests that the high internal body temperature induces an aberrant testicular hormone metabolism, still testoid, which might not be excreted as a 17-ketosteroid. Such aberrant metabolism induced by thermal change is postulated for androgenic activity of the ovary transplanted to the external ear of male mice (Hill, 1941).

For a study of pituitary stimulation it was thought advantageous to use animals which yielded very low normal 17–ketosteroid excretions since such animals might be pituitary deficient to a mild degree. It is also entirely possible that such animals are refractory to pituitary stimulation. Fraser et al. (1941) obtained increased excretion in hypogonadal patients showing primary pituitary deficiency with adminis-

tration of chorionic gonadotropin, while male castrates and eunuchoids with positive F.S.H. (gonadal deficient) showed no change. Possibly the dosages used were not sufficient to stimulate activity of the target organs since massive doses of purified adrenocorticotropic hormone have been found to increase 17–ketosteroid excretion in man (Mason et al., 1948; Forsham et al, 1948).

SUMMARY

Partially purified neutral ketonic fractions of rabbit urine were assayed for total 17-ketosteroid content by an anhydrous Zimmerman reaction. In the male rabbit castration produces an average decrease of 41% in the total urinary excretion of 17–ketosteroids. Experimental cryptrochidism depresses the normal 17-ketosteroid excretion to castrate level by thirty days. Subsequent cryptorchidectomy does not further decrease the 17-ketosteroid level. With moderate dosage, a polyvalent pituitary extract (Polyansyn, Armour) does not raise the 17-ketosteroid excretion of low-normal animals. Implantation of testosterone pellets into castrates causes a four-fold increase in 17-ketosteroid excretion sixteen days after implantation.

ACKNOWLEDGMENTS

I am very grateful to Dr. Boris Krichesky for advice and criticism during the progress of this study.

This work was aided by the University of California Board of Research, and through a Fellowship in Zoology granted by the University of California Graduate School. Crystalline hormones were generously supplied by Schering Corporation.

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PRESIDENTIAL ADDRESS AT THIRTIETH ANNUAL MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

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NEW HAVEN, CONNECTICUT

Members of the Association and their guests, ladies and gentlemen:

THE CONSTITUTION of the Association contains no provision that the President should address the members at their annual meeting, nor for that matter does it compel the membership to listen to him, yet the custom of our society has always been that this occasion is one at which the President is privileged to speak on matters pertaining to our common interest in endocrinology. In recent years your President's responsibility has been lightened by the fact that the address is usually delivered after the annual dinner and after a preliminary gathering which is nicely calculated to bring his audience into its most receptive mood. This adroit timing of the address not only enables the listeners to endure what is to follow but is in sharp contrast to the custom of another eminent medical society, which also once honored me with their presidency. Their barbarous custom was and is to compel the incumbent to address his unfortunate colleagues immediately after an early breakfast. For all concerned it was and is a chilling and disheartening experience. Indeed its effects are such as to remind me of the classical reply of the eminent British scientist who was invited to speak at a scientific meeting in this country. On his arrival he was informed by the secretary of the society concerned that his paper was scheduled for 7 A.M. the next day. Without a pause he replied "Young man I never stay up as late as that."

However, before I begin to speak on a more serious note I want to take this opportunity to convey to the members of the Association my sincere appreciation of the privilege that it has been to serve as your President during the past year. The presidency of this association is not only an honor but a responsibility. The honor is one I shall always cherish; the responsibility could not have been met had it not been for the cooperation of your Council and above all the hard work and tireless enthusiasm of the Secretary-Treasurer. The Association owes a deep debt of gratitude to Dr. Henry Turner and one that I consider it a privilege to acknowledge this evening. It is also

Received for publication June 21, 1948.

proper at this time to extend the appreciation and thanks of the Society to Dr. Willard Thompson and the other members of the local committee for the work they have done to make this meeting such a pleasant occasion for us all.

The question as to what I should devote the main body of my address this evening has given me no little concern. Judging from past years there were many possibilities ranging all the way from an interesting travelogue by one past president to rather detailed scientific papers by others. Since my peregrinations are of little interest and since we have a very full program of papers this year, it seemed more appropriate that I should spend a little time to discuss with you the past, present and, so far as one man can see, the future developments of endocrinology.

It is now thirty-one years since the first volume of ENDOCRINOLOGY was published and it is a salutary experience to re-read the papers presented in it, particularly if this is followed by a perusal of the volumes which were published in 1947. In volume I there appeared eighteen original articles, in the two volumes for 1947 a total of 126 original papers, to say nothing of those appearing in the *Journal of Clinical Endocrinology*. Yet these figures give a most inadequate picture of the changing character of this science. Among the sixteen papers in the first volume we note one by Brailsford Robertson on "Tethelin," the growth controlling constituent of the anterior pituitary, a principle stated to be active by mouth and stable at 80° C., a paper that drew cautious and guarded comment from Dr. Goetsch, for in those days the discussion of papers given at the Association meeting was printed in the journal. We also find in this first number the classical paper by Kendall on the isolation of thyroxine and its effects on human cretinism and myxoedema. But aside from these articles which have a flavor of modern experimental endocrinology, the remainder are essentially of a descriptive character. By this I mean they report the over-all effects of certain types of endocrine hypo- or hyperfunction in man and in reality represent the emergence of endocrinology from the world of giants, dwarfs, bearded ladies and the other oddities produced by extreme degrees of endocrine dysfunction with which it had for so long been identified.

I choose the term "emergence of endocrinology" with deliberation since it is my belief that we have now reached the stage when the functions of the endocrine glands are well enough understood to appreciate that they constitute a bodily system of which it is more important to know the basic knowledge of their mode of action and the minor aberrations of such function than it is to consider them only when they present themselves in such usually rare forms as Addison's disease, Cushing's syndrome, etc.

The emergence of endocrinology from the age of curiosities to a full fledged basic and clinical science has depended on three main

pathways of research. The first of these is the identification and isolation of the hormones themselves. In 1917 two hormones were clearly recognized and only one chemically identified. They were of course epinephrine and thyroxine; to these may be added the recognition of the activity of posterior lobe extracts. Today there has been isolated practically every known hormone, the majority in pure form. This phase of endocrinology may well be said to have been begun by the isolation of insulin in 1921, followed by the synthesis of thyroxine in 1926, the isolation of estrone in 1929, of testosterone and androsterone in 1935, the identification of the active adrenal cortical hormones as steroids in the early 1930's. The recognition that a protein, insulin, could also be a hormone led to a sustained attack on the other protein hormones, notably those of the anterior pituitary. Such success has now been achieved that the adrenotrophic, lactogenic, growth and gonadotrophic hormones have all been obtained in substantially pure form leaving only the thyrotrophic and possible other at present unidentified pituitary factors to the wiles of the protein chemist. Among the other protein hormones work still remains to be done on that of the parathyroid and certain gastrointestinal hormones.

These achievements carried out in several countries and by many different investigators have not only presented the physician with a remarkable armament of therapeutic agents but have led the way for the dissection of function of the endocrine system that is so essential for our understanding of its extraordinary effects on bodily processes. Their potency has also compelled a degree of caution in their use which is wholly desirable for medicine, for while our colleagues of thirty years ago may have fed anterior pituitary extracts with reckless abandon, no physician should, after what we have heard this afternoon, inject adrenotrophic hormone without careful consideration of its potent diabetogenic qualities. Indeed, I like to think that one of the important contributions of our meetings is to emphasize these qualities of present day endocrine preparations as much as to record our success in their isolation.

Therefore, so far as the isolation of the active principles of the known endocrine glands is concerned, it may be said to be substantially complete. The chemical structure of all except the protein hormones is rather exactly known; synthetic methods have been developed for epinephrine, thyroxine and many steroid hormones and are being continually increased. The contributions of the chemist to endocrinology in the last thirty years are perhaps the most outstanding event of this period.

The second line of endeavor that has enormously broadened our concept of endocrinology has been the parallel advances in our understanding of the intermediary metabolism of cells. This has included not only an analysis of the chemical steps involved but also the isolation and identification of the enzymes themselves. Not only have

we learned much about these but also the mechanism by which the chemical energy released by their action is made available for cellular function. In doing so, we have found the reason why certain vitamins are needed for the well being of the organism, for it is now known that these substances are necessary since they form parts of the enzyme systems whose activity underlies the whole of the processes of cellular activity. Since we are now agreed that the normal function of the hormones is to divert cellular processes to meet the circumstances imposed by changing conditions within or without the organism, the obvious next line of advance is to find the points of cellular metabolism upon which the hormones exert their characteristic effects.

In this regard, the belief has long been held that the hormones act only on intact cellular structures and were perhaps more in the nature of organisms of cellular activity than direct participants in cellular metabolic processes. Such a belief has now been shaken by the demonstration by Dr. and Mrs. Cori and their colleagues that one of the most important enzymes of carbohydrate metabolism—hexokinase—is inhibited by an anterior pituitary hormone and that this inhibition is removed by insulin; a demonstration of hormone action in an isolated system which gives at least a partial understanding of the effects of hypophysectomy in pancreatic diabetes and the production of diabetes by anterior lobe extracts.

We may expect within the next few decades or even sooner that the intimate relationship of hormones to cellular function will be as well understood as that of certain vitamins is today.

Now while the chemist has been supplying us with the information as to the chemical nature of the hormones and the students of metabolism have been uncovering the cellular mechanisms upon which they act, a third group of workers have been engaged upon another phase of endocrinology of no less importance that the former. This I choose to call the "integrative action of the endocrine system," paraphrasing the classical work of Sherrington on the nervous system. Such a term is of course more than a mere plagiarism since the endocrine system is the oldest correlating mechanism found in the higher forms of life. While it is true that the adrenal medulla and posterior pituitary are to be regarded as extensions of the autonomic nervous system, the remainder of the endocrine glands have so far as has been shown up to the present time only a slight dependence on the nervous system for the regulation and integration of their activity. The extensive research of the last thirty years has shown that such regulation and integration is largely achieved in two main ways. The first of these is the dependence of the gonads, the thyroid and the adrenal cortex on the anterior pituitary not only for the maintenance of their normal structural integrity but for their capacity to vary their characteristic secretions according to the needs of the body. This is

achieved as we all know by the elaboration and release of the specific trophic hormones from the anterior pituitary. There is in addition an inverse relationship between the activity of the target glands and their trophic hormones, inasmuch as a rise in the blood level of the hormone released from the target organ brings about a suppression of the secretion of its trophic hormone by the anterior pituitary. Such a delicately poised system while capable of rapid adjustment to bodily needs also offers many opportunities for the development of minor aberrations in function which if long continued may necessitate major alterations in the whole endocrine system and the processes they control. Such an example would be a decreased sensitivity of the anterior pituitary cells to the blood concentration of the target hormone, allowing in consequence abnormally high concentrations of the latter to reach the receptive cells for long periods.

The secretory activity of other endocrine glands notably the islets of Langerhans does not depend on the prior liberation of a trophic hormone but rather on the changing blood concentration of a metabolite. In the case of insulin this appears to be glucose and in the case of the parathyroids may be the plasma level of inorganic phosphate.

Thus the regulation and integration of the endocrine system involves at least three basic types of mechanism. First, a secretion that is a consequence of an increased degree of activity of the nervous system including both the voluntary and involuntary elements; second, one in which secretion is regulated by a delicate balance between the blood levels of the trophic and target gland hormones and, lastly, a simpler one in which the concentration of certain metabolites in the blood perfusing the gland are the regulatory factors. It is possible that various combinations of all these three may be involved in the operation of a single endocrine organ, particularly in the case of the anterior pituitary.

This then is a rough outline of our present understanding of the integration of the endocrine system. It is perhaps the most difficult problem in endocrinology but its complete understanding would enormously advance our ability to deal with endocrine disorders in man.

I would like to conclude my remarks this evening by some reference to what I consider to be the most important future lines for investigation in both experimental and clinical endocrinology. These are of course a personal selection but one I believe that would find the agreement of most endocrinologists.

The central position of the anterior pituitary both as a source of trophic hormones and those acting directly on the tissues makes it urgent that we should acquire much more information then we possess on the regulation of the secretory elements of this organ. There is as I have already indicated some evidence in the case of the trophic hormones that it is the blood level of the target hormone that is a

major factor. There is also some evidence in the case of the adrenotrophic hormone that the release of epinephrine by activation of the autonomic nervous system is a preliminary event. Yet we do not know the role, if any, of nerve elements that may reach these cells nor do we know how the selective discharge of pituitary hormones is affected. It appears unlikely at first sight that the release of F.S.H. and L. H. or of growth hormone and adrenotrophic hormone would occur simultaneously. Rather it would appear that the time sequence of discharge is adjusted in some manner to the circumstances prevailing in the body. Furthermore, we do not know whether in the period of rapid growth if the presumed excess of growth hormone in the blood is due to an increased secretion by the gland or a lowering of the rate of release of adrenotrophic hormone while maintaining a constant output of growth hormone. There are dozens of such questions relating to the function of the thyroid, gonads and adrenal cortex that require information about the regulation of anterior lobe secretion for their solution.

I have spoken several times of the importance of the blood levels of certain hormones as a determinant of endocrine activity. We are greatly handicapped in clinical endocrinology by our inability to find methods that will enable us to analyze or assay for such blood concentrations. Dependence on urine levels for the assessment of the secretory activity of an organ places us in the same position as were the clinicians of thirty years ago who had only urinary glucose measurements to guide their diagnosis and treatment of diabetes mellitus. We can anticipate an equally great increase in our ability to diagnose and treat endocrine disorders were we in a position to follow the blood levels of the various hormones.

Mention of diabetes mellitus calls to mind that perhaps with the exception of gonadal disturbances in the female that this is the most prevalent endocrine disorder encountered in man. Possibly two million people in this country suffer from an absolute or relative insufficiency of insulin secretion. While the isolation of insulin has enabled us to control the disease once it has presented itself, we are still largely in the dark as to the reasons for its onset. In no endocrine disorder is the collaboration of the student of intermediary metabolism and the endocrinologist more essential for the solution of the etiology of this disease. Much the same can be said for human hyperthyroidism. The advances in our understanding of thyroid physiology have been of a spectacular character and have led to the development of agents that can control hyperthyroidism by blocking out enzyme reactions essential for the formation of the hormone, yet here again little is known of the chain of events that lead to the clinical picture that ultimately presents itself for our relief.

A whole evening could be devoted to the shortcomings in our knowledge of gonadal dysfunction, particularly in the female. Replacement therapy at times brings spectacular results; in many others

it is quite incapable of re-initiating the normal sequence of events. Here perhaps as in no other situation is the coordinated and integrated activity of several endocrine glands an essential feature of normal physiological activity. A measure of our distance from the goal is to be seen in our failure to re-establish normal reproductive function in hypophysectomized animals.

I have always been interested in the comparatively small amount of attention that has been paid to the endocrinology and intermediary metabolism of pregnancy. Here is a physiological event quite evidently associated with widespread adjustments in the endocrine system and a sustained and additional burden on the processes of metabolism and yet I think it is fair to say that we know more of some rare endocrine disorders than we do of these events. Of equal interest, at least to me is the participation of certain hormones in the production of lactation. The mammary gland forms in its cells a unique protein, casein, a unique carbohydrate, lactose and fats of a type found only in milk. All these synthetic processes depend upon an adequate supply of the lactogenic hormone of the anterior pituitary and we would dearly like to know not only more about these unique metabolic reactions but also the relation of this hormone to them.

The present age finds the scientists of this country engaged in the most broad attack on the problems of malignancy that has yet been undertaken. Among the many fields that are being given particular attention is the relation of the steroid hormones, both to the genesis and treatment of certain types of malignancy. Promising results have been obtained by applying our knowledge of endocrinology to the treatment of malignancies arising in tissues that are known to be under hormonal control such as the breast and prostate. Others are investigating the hypothesis that aberrations of normal steroid metabolism may be the etiological basis of malignancy—a hypothesis that will not only require long and painstaking experimentation but most certainly necessitates an understanding of the effects of age, nutritive factors and the mechanism of normal hormone metabolism before decisive answers can be obtained.

I could continue to remind you for some time of the scantiness of our knowledge both in experimental and clinical endocrinology and one might despair of our ability to answer such questions were it not that contemplation of the advances in the thirty years since the establishment of the Association revives our hopes for the future. Thirty years ago endocrinology was struggling to be regarded as a respectable field of research; today this Association and its journals represent only a small part of the individuals who are working in this field, not perhaps calling themselves endocrinologists but appreciative of the fact that the capacity of the organism to regulate its function to its needs by means of hormones is vital to our understanding of normal and abnormal physiology.

In conclusion, I would like to say a few words to those physicians

whose problems lie in clinical endocrinology as it presents itself in their daily practice. Many have expressed to me their sense of frustration that the vast volume of experimental work appears at first sight to bear so little relation to human disease. I can only reply that these basic studies are essential, contradictory as they may often appear, yet the physicians should not forget that the contributions of the clinician have been and may be just as important as those from the laboratory. The names of Addison, Graves, Pierre Marie should remind us that careful observation and controlled studies in man are just as pertinent today as they were in their time. I regard it a privilege to have been the President of a society in which there is such a happy mingling of the laboratory worker and the clinician and I hope that the Association both in its membership and in its program will continue to reflect our joint interest in the problems of the function of the endocrine organs in health and disease.

MECHANISM OF INACTIVATION OF α-ESTRADIOL BY RAT LIVER "IN VITRO"[1]

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IT IS WELL known that the liver is the most important site of estrogen inactivation in different species. The early work of Zondek (1934) has shown that 95 per cent or more of the biological activity disappears when estrone is administered to man or rats or is incubated with rat liver "brei." Heller (1940) studied the inactivation of estrone, estradiol and estriol by slices of various tissues and the influence of several inhibitors upon this phenomenon. Engel and Rosenberg (1945) have observed that aqueous liver extracts, obtained at different pH, inactivate estrone in prolonged incubation. More recently Levy (1947), working with a liver extract prepared by a modification of Zondek's (1934) technique, has been able to study several properties of the inactivating system.

The present study was undertaken for the purpose of securing additional information regarding the nature of the estrogen-inactivating mechanism.

METHODS AND TECHNIQUES

Male Wistar descendants, weighing from 150 to 300 gm. were used throughout. Different types of liver preparations were employed. Rats were killed by a blow on the head and section of the neck with scissors. The liver was immediately removed and was either sliced according to the well known Warburg technique or subjected to the following procedures:

Three other preparations were used, i.e., frozen powder, homogenate and acetone powder.

(1) The powder was prepared by freezing liver with a mixture of dry ice and ethyl alcohol and pulverizing the hardened tissue in a mortar. A 10 per cent suspension of the powder in Krebs (1933) solution was used for the experiments.

(2) A 10 per cent homogenate was prepared. The required amount of liver was dropped in an ice-cold suspending medium, previously placed in the glass container of a Waring Blendor. The glass container was kept cold by packing with crushed ice and the tissue was homogenized for 3 minutes in a cold room at approximately 5° C.

Received for publication June 22, 1948.

[1] This work was supported by a grant-in-aid from the United States Public Health Service.

(3) The dry acetone powder was prepared according to the technique of Kaplan and Lipmann. The livers were rapidly removed and placed in an ice-packed dish. While cold, they were finely minced with scissors. The mince was transferred to the glass container of a Waring Blendor with 20 volumes of acetone, kept cold by packing with ice, and homogenized for two minutes in a cold room (5° C.). The homogenized material was allowed to settle for a few minutes, and filtered through a Buchner funnel of such size that the solid would make a layer about 1 cm. deep. It was not allowed to dry while filtering, and was washed twice with cold acetone and twice with peroxide-free dry ether. The washings were done at room temperature. The material was sucked dry and thoroughly dried in a vacuum desiccator over P_2O_5 for about an hour. The dry powder was passed through a 20 mesh sieve to remove connective tissue and stored in a stoppered bottle placed in a deep freeze chamber. When used, it was suspended in a convenient medium.

Three types of medium were used: (1) Krebs (1933) solution, (2) Fuhrman and Crismon (1944) "intracellular medium," and (3) 0.1 M sodium phosphate buffer, all at pH 7.4, buffered with phosphate.

The preparations, 100 mg. of slices, 10 per cent powder or homogenate or the equivalent amount of acetone powder, were incubated for two hours with constant shaking at 37.5° C. in 50 cc. Erlenmeyer vessels containing a total volume of 5 cc. with 1 or 2 μg. of α-estradiol per cc. A control, in which the α-estradiol was added at the end of the incubation period, was run in the same manner. An adequate amount of a solution of α-estradiol in 50 per cent ethyl alcohol containing 100 μg. per cc. was added to each vessel to attain the required concentration. After incubation, approximately 25 cc. of acetone were added to each vessel, the content of two vessels was pooled, and the biological activity determined, in castrated mice, by a modification of the Allen-Doisy technique (1923).

The error of assay was ±25 per cent when using 10 mice per dose and ±16 per cent for 20 mice per dose. For this reason only differences greater than 25 per cent were considered significant. The biological activity recovered after incubation was expressed in terms of the equivalent amount of α-estradiol.

In varying the conditions of incubation we have found that the several preparations used showed the same activity whether the gas phase was oxygen or air. For this reason, after the initial experiments, the aerobic incubations were carried out in air.

The boiled liver extracts used in some of the experiments were prepared by boiling liver mince, homogenate or liver slices in Krebs solution for 5 minutes, centrifuging and using the supernatant. In experiments with boiled mince and homogenate the amount of boiled extract equivalent to 0.5 gm. of liver was used in each vessel; with boiled slices extract the amount corresponded to 0.15 gm. of liver.

In the starvation experiments the rats were starved for 4 days.

The thiouracil treated rats received thiouracil in the drinking water (containing 0.5 mg. thiouracil per cc.) over a period of 60 days.

RESULTS AND COMMENT

Activity of Different Liver Preparations. The behavior of the liver preparations is shown in Table 1. Certain conclusions seem justified on the basis of these findings.

TABLE 1. INACTIVATION OF α-ESTRADIOL BY DIFFERENT MALE RAT LIVER PREPARATIONS

Preparation	Medium	No. of exper.	Initial α-estradiol	Recovery*	Inactivation
			γ	γ	%
Slices (control)	Krebs	4	10	10	0
Slices	Krebs	19	10	<3–0 (<0.7)	>70–100 (>93)
Slices	Krebs	10	20	<3–0.4 (<0.8)	>85–98 (>96)
Slices	Intracellular	2	20	0.8; 0.6 (0.7)	96; 97 (96.5)
Boiled slices	Krebs	2	10	10	0
Crushed frozen liver	Krebs	3	20	6–2 (3.7)	70–90 (82)
Homogenate (control)	Krebs	2	10	10; 8 (9)	0; 20 (10)
Homogenate	Krebs	13	10	8–3 (5.8)	20–70 (42)
Homogenate (control)	Krebs	2	20	20; 18 (19)	0; 10 (5)
Homogenate	Krebs	7	20	16–6 (8.9)	20–70 (55.5)
Homogenate (control)	Intracellular	2	20	18; 16 (17)	10; 20 (15)
Homogenate	Intracellular	2	20	6; 6 (6)	70; 70 (70)
Homogenate (control)	0.1 M phosphate buffer	8	10	10.3–6.4 (7.7)	0–36 (23)
Homogenate	0.1 M phosphate buffer	9	20	18–<6 (10.7)	10–>70 (46)
Homogenate supernatant	0.1 M phosphate buffer	2	20	>10; 6 (8)	<50; 70 (60)
Homogenate sediment	0.1 M phosphate buffer	2	20	12; 10 (11)	40; 50 (45)
Acetone powder (control)	Krebs	2	10	11; 8.8 (9.9)	0; 12 (6)
Acetone powder	0.1 M phosphate buffer	4	20	10–6.4 (8.6)	50–68 (57)
Acetone powder	Krebs	2	20	10; 6 (8)	50; 70 (60)

* Determined by bioassay and expressed as α-estradiol.
Note: Figures in parentheses indicate the average. In controls estradiol was added at end of incubation.

Fresh liver slices completely inactivated α-estradiol, while boiled slices had no inactivating effect.

The inactivation obtained with frozen pulverized liver, with the 10 per cent homogenate and with the acetone powder, was of the same order of magnitude, ranging from 20 to 90 per cent. No change in the behavior of the preparation was observed if the Fuhrman and Crismon (1944) "intracellular medium" was substituted for the Krebs (1933) solution or if 0.1 M sodium phosphate buffer pH 7.4 was used; addition of glucose had no effect.

The activity of the homogenate did not change appreciably after 24 hours dialysis at 5° C. against distilled water or against 0.1 M phosphate buffer pH 7.4.

The supernatant of the homogenate, obtained by centrifugation at 3000 r.p.m., showed the same activity as the original preparation. The sediment, brought to the initial volume, was less active.

Effect of Inhibitors. The action of several enzyme inhibitors on the activity of liver slices was tested with the purpose of gaining information regarding the properties of the inactivating system or systems.

The inactivation was completely inhibited when incubation was carried out in a nitrogen gas phase.

Several inhibitors were used and, as indicated in Table 2, only cyanide (70 per cent inactivation), azide (90 per cent inactivation) and capryl alcohol (30 per cent inactivation) exerted any appreciable inhibitory effect.

No inhibition of inactivation was observed in presence of malonate, monoiodoacetate, iodoacetamide, fluoride and thiourea.

Inactivation as a Dehydrogenation. Several experiments indicated that the inactivation of α-estradiol is at least partially accomplished by dehydrogenation.

TABLE 2. EFFECT OF INHIBITORS ON THE INACTIVATION OF α-ESTRADIOL BY MALE RAT LIVER SLICES

Inhibitor	Concentration	No. of exps.	Initial α-estradiol	Recovery*	Inactivation
			γ	γ	%
Cyanide	0.001 M	2	10	<3; <3 (<3)	>70
Cyanide	0.01 M	4	10	<3–3 (<3)	>70
Cyanide	0.02 M	1	10	1.5	85
Azide	0.01 M	1	10	<0.6	>94
Azide	0.02 M	1	10	<0.6	>94
Thiourea	0.001 M	1	10	<0.6	>94
Thiourea	0.01 M	2	10	(<0.6)	(>94)
Monoiodoacetate	0.002 M	2	10	<0.8; <0.4 (<0.6)	>92; >96 (>94)
Monoiodoacetate	0.01 M	1	10	<0.6	>94
Iodoacetamide	0.001 M	1	10	3	70
Iodoacetamide	0.01 M	1	10	<0.6	>94
Iodoacetamide	0.05 M	1	10	<0.6	>94
Fluoride	0.001 M	1	10	<3	>70
Fluoride	0.01 M	3	10	<3–<0.6 (<2.2)	>70–>94 (>78)
Fluoride	0.025 M	2	10	(<0.6)	(>94)
Fluoride	0.05 M	1	10	<0.6	>94
Malonate	0.01 M	1	10	<0.6	>94
Malonate	0.02 M	1	10	<0.6	>94
Caprylic alcohol	Saturation	2	10	8; 6 (7)	20; 40 (30)

* Expressed as α-estradiol determined by bioassay.
Note: Figures in parentheses indicate the average.

No inactivation occurred in a nitrogen gas phase. However, when methylene blue was added, about 60 per cent of the activity disappeared (Table 3). The degree of inactivation in the presence of methylene blue was not increased by addition of nicotinamide and DPN (a cozymase preparation, 60 per cent pure, obtained from Schwarz Laboratories).

The process of dehydrogenation in the presence of air is linked to DPN. This hypothesis is supported by several observations. In the first place, the addition of 10 to 20 mg. per vessel of nicotinamide and DPN, together or separately, to liver homogenate, increased the activity of the latter by approximately 50 per cent. No further increase was observed when cytochrome C[2] was added.

When the homogenate was prepared in the presence of 0.1 per cent nicotinamide, and DPN was added, there was a slight increase in activity over the preparation previously mentioned. Almost complete inactivation was achieved when the concentration of nicotinamide was increased to 2 per cent and 1.8 mM DPN was added.

Twenty-four-hour dialysis of the homogenate against distilled water or against 0.1 M sodium phosphate buffer pH 7.4, did not result in decrease in activity.

The addition of boiled liver extract, prepared from mince and homogenate as indicated above, to the homogenate did not increase its activity. However, the extract prepared from liver slices increased the activity of the homogenate by approximately 30 per cent, in agreement with the observation of Coppedge, Segaloff, Sarett and Altschul (1948).

Influence of Other Conditions. Citrate has been shown by Sweat and Samuels (1948) to increase the inactivation of testosterone by rat

[2] Obtained from Wyeth Institute for Biochemical Research.

TABLE 3. OBSERVATIONS SUGGESTING A DEHYDROGENATING MECHANISM FOR α-ESTRADIOL INACTIVATION BY MALE RAT LIVER

Preparation	No. of exper.	Gas phase	Methylene blue	Nicotinamide	DPN*	Cytochrome C	Initial α-estradiol	Recovery†	Inactivation
							γ	γ	%
Slices (control)	1	air	—	—	—	—	10	10	0
Slices	20	air	—	—	—	—	10	<3–0 (<0.7)	>70–100 (>93)
Slices	3	nitrogen	—	—	—	—	10	10–8 (9.1)	0–20 (9)
Slices	2	nitrogen	—	—	—	—	20	18; 15 (16.5)	10; 25 (17.5)
Slices	5	nitrogen	0.3 and 0.6 mM	—	—	—	10	6–3 (4.2)	40–70 (59)
Slices	2	nitrogen	0.6 mM	16.4 mM	1.8 mM	—	10	(4)	(60)
No tissue	1	nitrogen	0.6 mM	—	—	—	10	10	0
Homogenate (control)	2	air	—	—	—	—	10	8.6; 8 (8.3)	14; 20 (17)
Homogenate	2	air	—	—	—	—	10	6; 4.5 (5.3)	40; 55 (47)
Homogenate	5	air	—	—	—	—	20	16–6 (10.1)	20–70 (49.5)
Homogenate	2	air	—	10 mM	1.1 mM	—	10	5.4; 3 (4.2)	46–70 (58)
Homogenate	5	air	—	16.4 and 33 mM	1.8 and 3.6 mM	—	20	6–1.5 (4.7)	70–92 (76)
Homogenate	4	air	—	8.2 and 16.4 mM	0.9 and 1.8 mM	0.03 and 0.3 mM	20	4.5–4.5 (4.5)	77.5–77.5 (77.5)
Homogenate	1	air	—	16.4 mM	—	—	20	6	70
Homogenate	1	air	—	—	1.8 mM	—	10	2.2	78
Homogenate‡	1	air	—	—	—	—	20	>10	<50
Homogenate‡	2	air	—	—	1.8 mM	—	20	4.5; 3.6 (4.1)	77.5; 82 (80)
Homogenate§	1	air	—	—	—	—	20	6.6	67
Homogenate§	2	air	—	—	1.8 mM	—	20	2.3; 1.7 (2.0)	88; 91 (90)
No tissue	1	air	—	16.4 mM	1.8 mM	—	10	8.2	18

* Diphosphopyridinnucleotide.
† Determined by bioassay and expressed as α-estradiol.
‡ Prepared with 0.1 per cent nicotinamide.
§ Prepared with 2 per cent nicotinamide.
Note: Figures in parentheses indicate the average. In controls estradiol was added at the end of the incubation period.

liver. The addition of 0.001 to 0.005 M citrate to our type of homogenate did not increase the inactivation of α-estradiol (Table 4).

TABLE 4. INFLUENCE OF OTHER FACTORS IN α-ESTRADIOL INACTIVATION BY MALE RAT LIVER

Preparation	Effect of	No. of exp.	Initial α-estradiol	Recovery*	Inactivation
			γ	γ	%
Homogenate (control)	—	3	10	10.5–8.2 (9.6)	0–18 (6)
Homogenate	—	6	20	18–7.6 (11.4)	10–62 (43)
Homogenate	0.001 M citrate	3	20	11.4–6.4 (9)	43–68 (55)
Homogenate	0.002 M citrate	2	20	12.8; 9.5 (11.2)	36; 52 (44)
Homogenate	0.005 M citrate	5	20	12.3–10.8 (11.5)	38–46 (42)
Slices	Thiouracil treated rats	4	10	(<0.6)	(>94)
Homogenate (control)	Thiouracil treated rats	1	10	10	0
Homogenate	Thiouracil treated rats	4	20	8.2–6 (7.2)	59–70 (64)
Slices (control)	Starvation	1	10	12	0
Slices	Starvation	4	10	2.4–<0.6 (<1.3)	76–>94 (>87)
Slices (control)	0.43 mM phenol	1	20	20	0
Slices (control)	—	1	10	10	0
Slices	—	1	20	0.6	97
Slices	0.43 mM phenol	2	20	0.75; 0.3 (0.5)	98; 98.5 (97.5)

* Expressed as α-estradiol, determined by bioassay.
Note: Figures in the parentheses indicate the average.

With the idea that phenol might inhibit the inactivation, acting as a competitive substrate with the phenolic part of the α-estradiol molecule, we added 200 μg. of phenol per vessel (a concentration 10 times higher than that of the α-estradiol used). No effect on the inactivation was observed.

The livers of thiouracil-treated rats inactivated α-estradiol to the same extent as those of the normal rats.

A slight decrease in activity was noticed in livers of rats starved for 4 days; further experiments will be necessary to confirm this observation.

CONCLUSIONS

As shown by previous workers, liver slices (Heller, 1940) and "brei" (Zondek, 1934) inactivate estrogens. The process is enzymatic, inasmuch as boiled liver slices show no such activity. The transformation seems to be oxidative in nature as it does not occur in a nitrogen gas phase. That a dehydrogenating mechanism is at least partially involved in the process of inactivation is indicated by the observation that addition of methylene blue reestablishes in part the activity of slices incubated anaerobically. We believe that methylene blue acts as a hydrogen acceptor and that, under these conditions, estradiol may possibly be converted to estrone.

The dehydrogenation is in part linked to DPN. This is indicated by the increase in activity of the homogenate after addition of DPN and by the augmenting effect of nicotinamide. The latter probably produces this effect by decreasing the breakdown of DPN, as it has been shown by Mann and Quastel (1941) and Handler and Klein

(1942) to inhibit the breakdown of this coenzyme by different tissue preparations. This property may explain the great difference observed between the activity of slices and the other types of preparation, i.e., frozen powder, homogenate, acetone powder. Hydrolysis of the products of incubation produces no increase in recoverable activity. This suggests that conjugation has not occurred, which is in agreement with the findings of Heller (1940). However, in view of the error inherent in the assay procedure, this possibility cannot be entirely ruled out inasmuch as Zondek (1934) by hydrolysis of the carcass of rats, was able to recover 20 per cent of the estrogenic activity of the estrone administered during the preceding 24 hours.

The rather slight inhibition of inactivation produced by cyanide and azide, contradicting the observations of Heller (1940) and Levy (1947), indicates that the system cytochrome-cytochrome oxidase does not participate to a large extent in the process of inactivation under the conditions of our experiments.

The effect of the various inhibitors studied indicates that heavy metal and calcium-containing enzymes, -SH enzymes and succinic acid dehydrogenase are not involved in the hepatic inactivation of α-estradiol in vitro.

SUMMARY

The inactivation of α-estradiol by different types of male rat liver preparation has been studied. A 2-hour incubation was carried out in 5 cc. of medium (Krebs solution, Fuhrman and Crismon "intracellular" or 0.1 M sodium phosphate buffer, all buffered with phosphate at pH 7.4) at 37.5° C. Usually 1 or 2 μg. of α-estradiol per cc. was used. The amount of biologically active material present at the end was determined by bioassay in spayed mice.

Complete inactivation by liver slices was observed in oxygen or air. No inactivation took place in nitrogen or with boiled slices.

The 10 per cent frozen liver powder, homogenate or the equivalent amount of acetone powder produced 20 to 90 per cent inactivation. Neither the type of medium used nor the presence of glucose influenced these results.

The activity of the homogenate was not changed appreciably by 24 hours dialysis. Boiled liver extract prepared from mince or homogenate did not increase the activity of the homogenate or dialysate, but the boiled extract obtained from fresh liver slices produced a detectable increase.

Centrifugation of the homogenate at 3000 r.p.m. gave a supernatant of the same activity, but the sediment brought to the initial volume was less active.

A slight degree of inhibition of inactivation was observed in the presence of cyanide and azide (70 to 90 per cent inactivation). Only 30 per cent inactivation occurred in the presence of capryl alcohol. No

inhibition was observed in the presence of malonate, monoiodoacetate, iodoacetamide, fluoride and thiourea.

The addition of methylene blue to slices incubated in nitrogen brought about 60 per cent inactivation.

Nicotinamide and DPN added to the homogenate increased the inactivation by approximately 50 per cent; no further increase was observed when cytochrome C was added. Homogenate prepared with nicotinamide showed greater activity than that obtained in its absence.

Citrate, phenol and the feeding of thiouracil had no effect on estradiol inactivation. A slight decrease was noticed when the rats were starved for 4 days.

ACKNOWLEDGMENTS

We are indebted to Dr. E. Schwenk of the Schering Corporation and Drs. E. Oppenheimer and C. R. Scholz of the Ciba Pharmaceutical Products, Inc. for material used in this study, to Dr. E. S. G. Barron for the iodoacetamide and to Dr. J. Seifter for the Cytochrome C preparation and for technical assistance to Miss Dorothy Ozer and Miss Ann Roberts.

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THE ANTITHYROID EFFECT OF CERTAIN FOODS IN MAN AS DETERMINED WITH RADIOACTIVE IODINE[1]

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ALTHOUGH iodine deficiency is well established as an etiologic factor in endemic goiter, it sometimes appears that other agents may be responsible. It is evident that the sporadic goiter which occurs in a "goiter-free" area where there is a relatively high concentration of iodine in the soil and water cannot be satisfactorily explained on an iodine deficiency basis.

Before the discovery of iodine, investigators had advanced many hypotheses as to the cause of goiter, but with the general acceptance of the "iodine-lack" theory in the second and third decades of this century most of the earlier ideas were discarded. In view of the inability of this "deficiency" theory to explain many instances of this disease, however, other causes have been sought.

For many centuries the thought has existed that dietary habits might be responsible for endemic goiter. The Tyroleans, for instance, believed that their "swelled necks" were caused by eating a type of Alpine chestnut (Barton, 1800). Around the turn of the last century several investigators claimed to have shown a goitrogenic effect of certain foods in laboratory animals, but it was not until 1928 that interest was truly stimulated in the possibility that food might be a cause of this disease. In that year, Chesney, Clawson, and Webster (1928) reported that a colony of rabbits they were maintaining for work in experimental syphilis developed large goiters while on a daily ration of cabbage. After considering other factors which might have been responsible, these workers concluded that the cabbage per se was the etiologic agent. In other words, there was a positive effect exerted by this vegetable which caused thyroid enlargement in spite of an adequate iodine intake.

This experiment has been confirmed several times in the twenty years that have followed. Since the original demonstration of activity in cabbage, a variety of foods, including chard, rape, turnip (Hercus

Received for publication June 22, 1948.

[1] Aided by grants from the United States Public Health Service and from the American Cyanamid Company.

and Purves, 1936), soybeans (Sharpless, 1938, McCarrison, 1934), and peanuts (McCarrison, 1937), has been claimed to cause goiter in both laboratory animals and livestock.

Since cabbage is a member of the family Cruciferae, it is not strange that the majority of the investigations has been carried out on this group. This family is generally thought to possess stronger goitrogenic potency than other plants, with the genus Brassica (containing cabbage) seemingly its most active subgroup. Hercus and Purves (1936) have also shown that Brassica seeds produce considerable thyroid hyperplasia in the rat. Most of the search for goitrogenous food has been confined to plant derivatives, but some animal products have also been incriminated, among them fats and liver.

The active agent responsible for the goitrogenic effect of these foodstuffs has not been identified with certainty as yet. When it seemed apparent that most of the Brassicae caused thyroid hyperplasia, it was postulated by Marine et al. (1931) that the mustard oils (isothiocyanates), the most characteristic compounds of this genus, were the ingredients involved. These, however, proved inactive when tested in the rat. After trying several other compounds contained in these plants, Marine's group found that the cyanides were quite goitrogenic, acetonitrile being the most active.

Many workers have been unable to confirm this action of cyanide, however. With the discovery of the goitrogenic properties of thiourea and related compounds, it was postulated that the active substance in foods might well be one of this group. Since it was known that the mustard oils could be converted by ammonia to thioureas, allylthiourea was suggested by Kennedy as a possibility. As yet, however, attempts at isolation of an active goitrogenic compound from foodstuffs have failed.

Thiocyanate has also been shown to cause thyroid enlargement (Barker, 1936). It has now been well demonstrated that the action of this substance is to prevent the selective concentration of the iodide ion in the thyroid and to lead to the development of goiter if the iodine intake is sufficiently low. The organic binding of iodine seems to be unaffected (Wolf et al., 1946, VanderLaan and VanderLaan, 1947). Thiocyanate, therefore, must be added to the list of possibilities.

A serious drawback in attempting to interpret the results of these animal experiments in their relation to the pathogenesis of goiter in man is the marked species difference which exists in the response to antithyroid agents. It would be instructive to assay various foodstuffs directly on human subjects; but until quite recently this has been impractical. However, the determination of antithyroid activity by observing the inhibition of the radioactive iodine uptake (Stanley and Astwood, 1947) was found to provide a rapid and convenient method for assaying the effect of various foods in man. In this paper the results of testing a number of the more common foods by this method are reported.

METHODS

The subjects were technicians, medical students, and physicians. They were asked to omit breakfast and at 9 A.M. they were given a tracer dose of 100 microcuries of radioactive I^{131} in 10 cc. of physiological saline. This was followed by 10 cc. of tap water to wash the remaining iodine into the stomach from the flask, mouth, and pharynx. The uptake of radioactive iodine by the thyroid was then determined, as previously described, by serial counts taken over the thyroid isthmus with a shielded Geiger-Muller counter. A brass cap was placed over the window and in contact with the skin so that only gamma radiations were recorded.

By plotting the number of counts per second against the square root of the time in minutes, a straight-line relationship was found to exist with normally functioning thyroid glands. This relation was maintained quite well over a period of eight to twelve hours, except in those subjects which exhibited unusually rapid rates of uptake; in these instances there was a tendency for the points to fall below such a line towards the end of the day. The slope of this line has been termed the "accumulation gradient." Any antithyroid effect could be detected by the deviation of the gradient so that the plotted points were below where they would be if the straight-line relationship continued to hold.

After the ingestion of the I^{131}, counts were taken about every fifteen minutes until the course of the uptake seemed to be fairly well established. This usually required sixty to ninety minutes. The subject was then given the food to be assayed and instructed to eat to satiety in as short a time as possible. The total period of ingestion varied from one minute to two hours, the average being approximately forty minutes. Following the completion of the test meal, the subjects were free to go about their duties, returning for counts at one-hour intervals during the day. The final count was usually made between 4:30 and 5:00 P.M. In order to ascertain the effect of the test meal over as long a period as possible, the determinations on nine subjects were continued until 8:30 to 9:00 P.M. Following the final reading on the day of the test, the subjects were permitted to eat any food they desired but were asked to keep a list of all the material ingested (including liquids). A final reading was taken the next morning between 10:00 and 10:30 A.M. Except for a little coffee, tea, or water, no food was taken other than the test meal on the day of the experiment until after the final count in the afternoon.

The degree of inhibition of iodine uptake was arbitrarily graded in the following manner:

0—No inhibition. The course of the iodine accumulation did not deviate significantly from prediction.

1—Slight or questionable depression of the rate of uptake.

2—Moderate deviation from prediction but without complete inhibition.

3—Complete inhibition of the iodine uptake but for less than four hours.

4—Complete inhibition of uptake lasting more than four but less than twenty-four hours.

5—Complete inhibition of the iodine uptake lasting twenty-four hours.

The amount of radioactive iodine in the gland at the twenty-five-hour counting was compared with a graph which correlated the twenty-five-hour reading with the accumulation gradient of the preceding day in untreated normal subjects (Figure 1). The correlation seemed to hold fairly well within

relatively narrow limits in normal individuals. In the food experiments, a marked inhibition on the day of the test was often followed by a twenty-five-hour reading well below the average according to this graph. A slight or moderate depression of the gradient would often have no effect on the radioactivity present in the gland the next day.

It soon became apparent, however, that the twenty-five-hour count would sometimes be depressed although there had been no apparent antithyroid effect during the first day. Those who had a depression of their

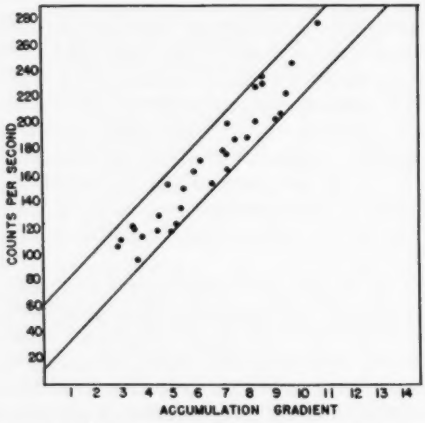

FIG. 1. The relation between the accumulation gradient on the first day of iodine uptake studies in normal, untreated subjects and the 25-hour count.

twenty-five-hour count without any apparent effect the day before had sometimes consumed considerable quantities of food suspected of having antithyroid properties. Consequently, the twenty-five-hour reading was of little value in assessing the degree of effect in these tests and was used only to differentiate between a grade 4 and 5 effect.

The test meal was limited to a single foodstuff in all except one of the experiments. No condiment was given during the first few tests; but, after it was established that it had no effect, table salt was used. A little butter was allowed with some of the cooked vegetables.

RESULTS

The following table lists the various foods tested and the grading of their effect by the arbitrary classification given above. There are three broad categories: Animal, Vegetable, and Mineral.

The products of plant origin are listed under their family names, arranged in alphabetic order. The members of the various families are listed in the first column alphabetically under their common names, with the scientific names in the second column. As the animal group consists of only a few members, these are listed only in the alphabetic order of their common names.

A total of 61 foods were tested in 100 different subjects. Most of those which showed more than a slight effect were tested at least twice, and one (rutabaga) was tried seven times. The subjects ingested the material in the raw state, when possible, since it was felt that activity might be destroyed by heat. If the raw foodstuff showed activity, it

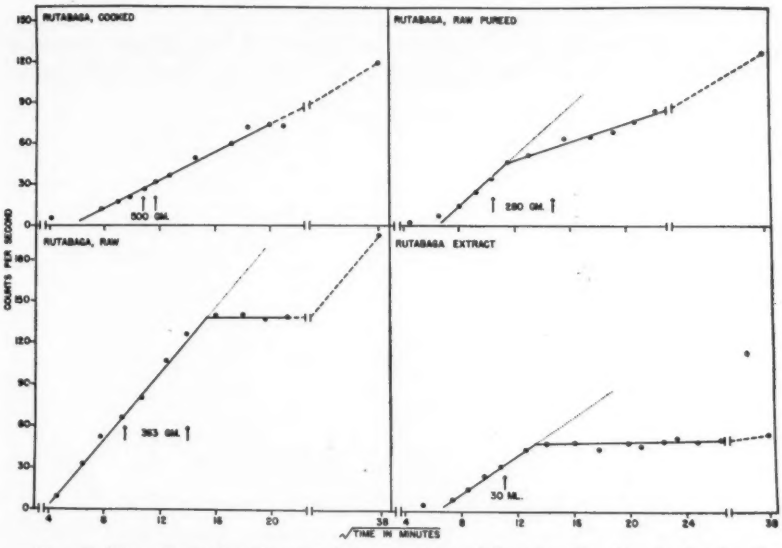

FIG. 2. The effect of eating rutabaga on the uptake of radioactive iodine by the thyroid. Cooked rutabaga had no effect. 280 gm. of the raw, pureed vegetable had a grade 2 effect. 363 gm. of raw chunks had a grade 3 effect. 30 cc. of a purified extract equivalent to 2617 gm. inhibited the uptake completely for 24 hours.

was usually tried after cooking, also. Some of the foods are so unpalatable in an uncooked condition, however, that no one would volunteer to eat them. Similarly, no one seemed anxious to try certain materials, such as lettuce, after cooking.

Since most of the positive goitrogenic effects shown by previous animal experiments seemed to be given by the Cruciferae, more tests were made among members of this group than of any other. Contrary to expectation, a high degree of activity was not found in cabbage. However, another member of the mustard family, rutabaga, was uniformly inhibitory. This vegetable has consistently given the strongest antithyroid effect. Turnips, closely related to rutabaga, possessed considerable activity also, but not to as marked a degree. The other members of the Cruciferae which were tested have so far proved inactive.

It is of interest that a definite inhibitory effect could be obtained from cabbage in only one instance. Since the goitrogenicity of this food has been quite definitely established for rats and rabbits, the

lack of a consistent effect on human beings may mean either that a considerable species difference exists in the response to this material or that the cabbage we obtained was relatively impotent.

Of the other families tested, the Chenopodiaceae, Compositae, Cupuliferae, Juglandaceae, Leguminosae, Rosaceae, Rutaceae, Umbelliferae, and Vitaceae showed definite evidence of activity among certain of their members.

Chenopodiaceae. All the tested members of this family produced inhibition of the uptake of iodine by the thyroid. Beets and spinach

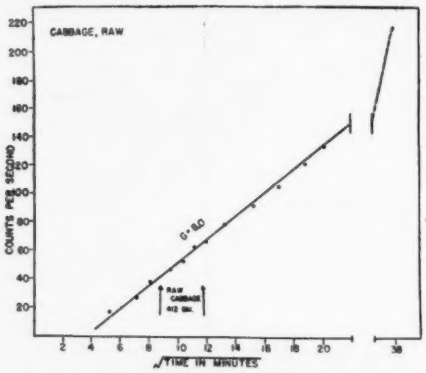

FIG. 3. The lack of effect of raw cabbage on the iodine uptake.

were both employed in the cooked state. In the one negative test given by spinach, only 322 gm. were eaten. Since this was less than half the amount consumed in the two positive tests of this foodstuff, the lack of effect in this instance might well be due to an insufficient concentration of the active principle in the body fluids.

Compositae. Raw lettuce, the only one of this group tested, had a slight effect in two instances.

Cruciferae. As previously mentioned, the most active food we found, rutabaga, is a member of this family. This vegetable possessed marked antithyroid activity when ingested in the raw state in amounts varying from 280 to 363 grams. We were unable to obtain any effect when the rutabaga was cooked, however, other than a

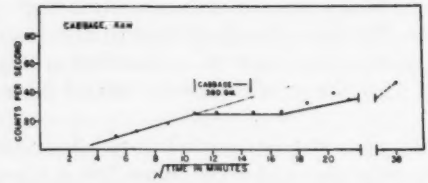

FIG. 4. This grade 3 inhibition was the only definite effect obtained from cabbage.

slight inhibition when 515 grams were consumed. The raw rutabaga was effective when eaten whole, as a puree, or when only the pressed, filtered juice was used. This indicated that the active principle was probably water-soluble. As a further confirmatory procedure, 30 cc. of a purified rutabaga extract, equivalent to 2617 gm. of the raw food, were taken by one subject. This resulted in a complete inhibition of the iodine uptake which lasted for twenty-four hours.

Cooked broccoli and cauliflower showed no effect, nor did dry mustard in the small quantity of 0.9 gm. Radish also proved impo-

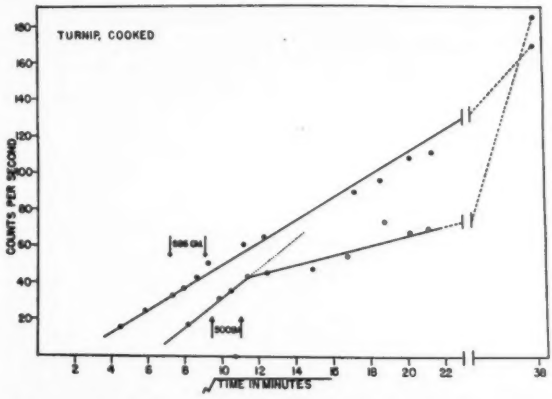

FIG. 5. The inconsistent effect of cooking. 686 gm. of cooked turnip did not inhibit the iodine uptake whereas a smaller meal of 500 gm. had a grade 2 effect.

tent. Raw turnip was definitely inhibitory in a dose of 441 gm. but had no effect when half that amount was taken. Cooked turnip depressed the uptake in one test when 500 gm. were eaten but had no effect in two other tests with 514 and 686 gm.

The largely negative findings in the case of cabbage have already been discussed.

Cupuliferae. The finding that only 186 gm. of raw filberts exerted a demonstrable inhibition suggests that this nut may possess a considerable degree of antithyroid activity. Unfortunately, we were unable to find a volunteer who could consume a larger quantity.

Juglandaceae. Walnuts showed evidence of activity comparable to that of the filberts.

Leguminosae. Several members of this group were found active: baked beans, string beans, peas, and peanuts. Cooking seemed to destroy the activity of the string beans, while it had no effect on that of the peas or peanuts. It is interesting that the baked beans were the only canned product in which we were able to demonstrate any activity. Black beans and lima beans were the only legumes tested which had no effect.

Rosaceae. The rose family also possessed active members. Raw pears and filtered pear juice showed moderate antithyroid activity, but no effect was obtained from canned pears. The latter, however, were ingested in a much smaller quantity. An alcoholic extract of pears, equivalent to 4400 gm. showed only a questionable degree of inhibition. Other evidence also indicated that alcoholic extraction was not an efficient method of obtaining the active principle.

The first test made on strawberries was quite striking. There was a complete inhibition of the iodine uptake which was maintained

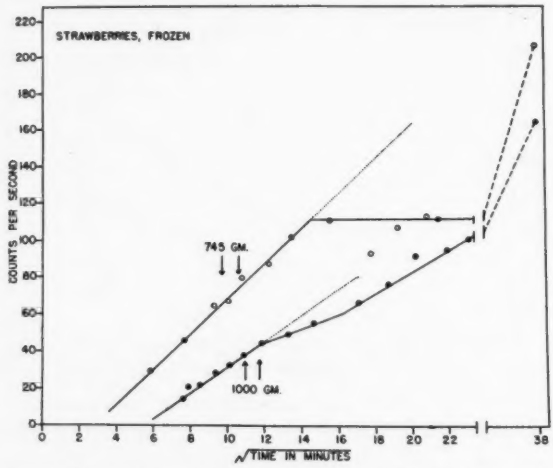

FIG. 6. 745 gm. of strawberries exerted a grade 4 inhibition in one subject while 1000 gm. of another batch had only a grade 2 effect.

until the final reading on the first day. However, the three subsequent tests of this product were not in agreement with the first. Two of these showed only a slight effect, while the third was negative. In each instance approximately the same quantity of strawberries was ingested. These differences in results may have been due either to a difference in the potency of the lots of fruit, or to a remarkable susceptibility of the completely inhibited subject. However, the previous test meal this subject had eaten (onions) had had a complete lack of effect on the accumulation gradient.

Raspberries, loganberries, and blackberries were inactive in the quantities tested, as were almond and apple. Peaches showed a definite effect, while apricots were only slightly inhibitory.

Rutaceae. Orange juice and grape.ruit were moderately inhibitory but were taken in very large amounts. Tangerines had no activity.

Umbelliferae. Carrots proved quite active when eaten in adequate

quantity. A negative effect was obtained in only one of six instances from this foodstuff, and the 207 grams consumed in this test was approximately half that eaten in the other carrot experiments. Celery was responsible for a short period of complete inhibition when 630 gm. were eaten while 396 gm. had no effect.

Vitaceae. Fresh grapes had no activity, but when eaten in the form of raisins they caused deviation of the gradient although less than one-third of the amount taken fresh was consumed. This difference in potency was most likely due to the dissimilarities of the varieties used. The fresh grapes were Tokay, while the raisins were Thompson seedless.

Animal. Of the products tested which can be classified as animal, milk had the most consistent antithyroid effect. The inhibition produced was never marked but seemed definite, both as pasteurized

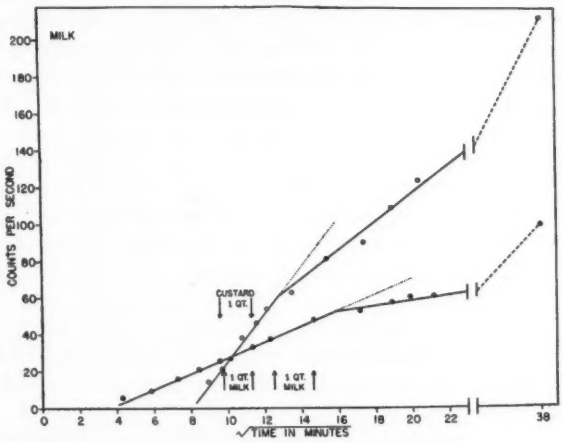

FIG. 7. Cow's milk, either as baked custard or as uncooked, pasteurized milk, gave a grade 2 inhibition.

milk and as baked custard. Of the five tests carried out on milk or milk products, only two negative results were obtained. One of these was with processed American cheese where only a small quantity (160 gm.) was consumed. The other negative test in this group followed the consumption of three quarts of pasteurized milk. Since this would seem to be an adequate amount, one can only state that milk, as such, had a positive effect in two out of three instances. The active principle which may be contained in this food is apparently heat-stable as neither baking nor pasteurization seemed to destroy the antithyroid effect.

Raw oysters and raw clams also depressed the iodine uptake. No other seafood tested showed any such inhibition. While it is possible

that the depression of the accumulation gradient by oysters and clams may have been due to the high-iodine content they are known to possess, the absence of activity from the other iodine-rich seafood is difficult to correlate with such an hypothesis. It is possible, of course, that boiling a marine animal will leach out enough iodine to reduce the concentration of this element below an effective level. In line with this thought, it might be noted that the oysters and clams were the only ocean residents eaten raw.

Because several observers (Remington, 1937, Hou, 1940) have indicated that liver may be a potent goitrogen in the rat, this substance was tested in man also. As yet only one such test has been made. One pound of broiled beef liver gave evidence of a definite, though slight, thyroid inhibition.

Mineral. In some of the tests with a grade 1 inhibition it was rather difficult to state categorically whether or not the effect was significant. Because it was possible that some of these questionable effects might be due to inert materials, a test was made to determine what result would follow the administration of a large amount of salt. 28.4 gm. of sodium chloride were given orally with no demonstrable depression of the iodine uptake. This result indicated that any possible displacement of iodide by the chloride ion had no significance in our tests as no meal given had a salt content which approached this amount. Since three liters of water would have to be drunk by this subject to maintain isotonicity of the body fluids, this test also indicated the lack of inhibition by a large, inert fluid intake.

Mixed Food. Since it seemed unlikely that meals would very frequently be limited to a single food, a test was made which combined carrots, rutabaga, lettuce, pears, and milk. This resulted in a 1–2 degree inhibition, indicating that such combinations could also have antithyroid activity.

DISCUSSION

These results suggest that there are materials in certain foods which are capable of inhibiting the uptake of iodine by the human thyroid. Of the products thus far tested, rutabaga consistently possessed greater activity than any other. On the whole, those foods belonging to the vegetable kingdom seem more active than animal products.

Some of the tests which yielded only grade 1 effects were difficult to interpret and probably were of no significance; however, those that were stronger than this probably represent a true inhibition of the thyroid. Distention of the stomach by a bulky mass or dilution of the body fluids by the addition of large amounts of liquid probably did not significantly alter the rate of thyroid iodine accumulation. For instance, the three quarts of milk and the 1263 gm. of banana that were ingested had no effect although they were as large in quantity as any other food tested. The negative effect from sodium

chloride previously cited would lend support to such an inference.

Although rutabaga had the strongest antithyroid effect, activity was by no means limited to the Cruciferae. Among the Rosaceae, for instance, pears, strawberries, and peaches seemed to be quite active. Whether the material responsible for this effect was the same in each instance or whether, as seems more likely, different compounds were responsible in different foods can only be answered by the isolation and indentification of the active principle in each case.

It is curious that the activity of some foods, e.g. rutabaga and pears, seemed to be destroyed by cooking while the activity of others, such as peas and peanuts, was unaffected by heat. Since the antithyroid ingredient seemed to be water soluble, perhaps the heating of some of these foods in water extracted a good proportion of the activity. The cooking juices were usually not eaten in these tests so the material ingested may have thus lost a good deal of its inhibiting power. Peanuts, on the other hand, were roasted and there was therefore no opportunity for the activity to be extracted.

Of course, some of the vegetables which retained their activity when cooked were also heated in water. In these cases there might conceivably be a difference in solubility of the active material or some mechanical interference with the water's ability to reach the food pulp. Peas and beets, for example, were cooked whole and the integrity of the outer coat might have at least partially obstructed the access of water to the interior. In other instances where such an explanation could not hold, the juices may have been eaten as well as the pulp. It is also possible that if the goitrogens in different foods are not the same, heat may destroy some and not others.

A pertinent question is whether the iodine content of the active foods contributed to the depressed rate of accumulation of radioactive iodine. Tests with various doses of potassium iodide have shown that approximately 1 mg. would be required to induce a perceptible effect. Although the iodine content of the foods used in these experiments was not determined, the extensive literature on the subject indicates that most of the test meals were very low in iodine. The sea foods were an exception and the values quoted by McClendon (1939) indicate that several milligrams of iodine may have been present. The fact that positive effects were observed only with raw oysters and clams might indicate that the iodine present in the other sea foods was not in the form of iodide or not in a form which was readily available for absorption by the thyroid. Another possibility, that boiling might leach out the iodide from the sea food, has already been mentioned.

The findings reported here appear to have some significance in relation to the development of simple goiter. If the diet consistently contained foods high in antithyroid activity, it is possible that goiter would eventually result. A planned experiment to test this possibility has not been tried, but cases have been observed of goiter

		State	Quantity	Effect
		ANIMAL		
Beefsteak		raw	448	0
Bonita		canned	308	0
Cheese		American, pasteurized	160	0
Clams	Venus mercenaria	raw	2 dozen	1
Custard		baked	1000	1
Ice cream		frozen	1 quart	0
Liver		broiled	1 pound	1
Lobster	Homarus sp.	boiled	600	0
Milk		pasteurized	2 quarts	1
Milk		pasteurized	2 quarts	2
Milk		pasteurized	3 quarts	0
Oysters	Ostrea virginica	raw	1 pint	2
Oysters		raw	1 pint	0
Sardines		canned	218	0
Shrimp	Crangon sp.	boiled	400	0
		AGARICACEAE		
Mushrooms	Agaricus campestris	cooked	570	0
		ARECACAE		
Date	Phoenix dactylifera	dried	400	0
		BROMELLIACEAE		
Pineapple	Ananas comosus	canned	1200	0
		CHENOPODIACEAE		
Beets	Beta vulgaris	cooked	502	1
Spinach	Spinacia oleracea	cooked	322	0
Spinach		cooked	808	2
Spinach		cooked	900	1
		COMPOSITAE		
Lettuce	Lactuca sativa	raw	284	1–2
Lettuce		raw	299	1
		CRUCIFERAE		
Broccoli	Brassica oleracea botrytis	cooked	263	0
Cabbage	Brassica oleracea	raw	260	0
Cabbage		raw	412	0
Cabbage		raw	380	1
Cabbage		raw	380	3
Cauliflower	Brassica oleracea botrytis cauliflora	cooked	560	0
Mustard	Brassica alba	dry	.9	0
Radish	Raphanus sativus	raw	358	0
Rutabaga	Brassica napobrassica	raw	363	3–4
Rutabaga		raw, pureed	280	2
Rutabaga		juice, raw	313	2
Rutabaga		purified extract	30 cc. (equiv. 2617 gm.)	5
Rutabaga		cooked	500	0
Rutabaga		cooked	515	1
Rutabaga		cooked	290	0
Turnip	Brassica rapa	raw	234	0
Turnip		raw	441	2
Turnip		cooked	514	0
Turnip		cooked	686	0
Turnip		cooked	500	2

		State	Quantity	Effect
		CUCURBITACEAE		
Cucumber	Cucumis sativus	raw	590	0
Honeydew		green, frozen	456	1
Squash, banana	Cucurbita maxima	cooked	400	0
		CUPULIFERAE		
Filbert	Corylus avellana	raw	186	1
		GRAMINEAE		
Corn	Zea Mays	frozen	475	0
Rice	Oryza sativa	boiled	350	0
Rye	Secale cereale	bread	250	0
		JUGLANDACEAE		
Walnut	Juglans regia	raw	330	1–2
		LEGUMINOSAE		
Beans, black	Dolichos lablab	cooked	334	0
Beans, baked		canned	479	1
Beans, lima	Phaseolus limensis	cooked	290	0
Beans, string	Phaseolus vulgaris	raw	389	1–2
Beans, string		cooked	633	0
Peas	Pisum sativum	raw	880	1–2
Peas		cooked	443	1–2
Peanut	Arachis hypogaea	raw	200	1
Peanut		roasted	218	1
		LILIACEAE		
Onion	Allium cepa	raw	93	0
		OLEACEAE		
Olive	Olea europaea	pickled, green	200	0
		ROSACEAE		
Almond	Prunus Amygdalus	raw	132	0
Apple	Malus pumila	raw	689	0
Apricot	Prunus Armeniaca	dried	320	1
Blackberry	Rubus spp.	frozen, raw	825	0
Loganberry	Rubus loganobaccus	frozen, raw	761	0
Peach	Prunus Persica	frozen, raw	810	2
Pear	Pyrus communis	raw	1455	2
Pear		juice, raw	803	2
Pear		raw	1044	1
Pear		canned	530	0
Pear		alcoholic extract	equiv. 4.4 kg.	1
Raspberry	Rubus idacus	frozen, raw	447	0
Strawberry	Fragaria spp.	frozen, raw	746	5
Strawberry		frozen, raw	860	0
Strawberry		frozen, raw	1000	1
Strawberry		fresh	791	1
		RUTACEAE		
Grapefruit	Citrus grandis	raw	1326	2
Orange	Citrus aurantium	juice, raw	1179	1
Tangerines	Citrus reticulata	raw	430	0

		State	Quantity	Effect
		SCITAMINAE		
Banana	Musa sapientum	raw	1263	0
		SOLANACEAE		
Green pepper	Capsicum annuum var.	raw	244	1
Potato	Solanum tuberosum	raw	381	0
Potato		boiled	692	0
Tomato	Lycopersicum esculentum	raw	600	0
Tomato		juice, fresh	855	0
		UMBELLIFERAE		
Carrot	Daucus carrota	raw	416	1
Carrot		raw	595	1–2
Carrot		raw	378	2–3
Carrot		raw	352	3
Carrot		raw	207	0
Carrot		cooked	554	1
Celery	Apium aveolengrs	raw	630	3
Celery		raw	396	0
		VITACAE		
Grapes	Vitis vinifera	fresh	680	0
Grapes		dried	275	1–2
		MIXED MEAL		
Carrots, rutabaga, lettuce		raw	420	1–2
Pears		raw	311	
Milk		pasteurized	3 glasses	
		MINERAL		
Sodium chloride		tablet	28.4	0

developing in patients who were vegetarians or who suddenly had cravings for certain foods, such as lettuce. Certainly it seems indisputable that goiter can be caused in animals by a high dietary intake of certain foodstuffs. Not only is this true in the laboratory, but also among livestock, as in the epidemics described by Hercus and Purves of goiter in sheep which were fed on turnip during the winter.

Of course, the amount of food consumed in the above experiments was unphysiologically large in most instances. However, some foods, such as peanuts, rutabagas, and filberts, exerted an inhibition when relatively small quantities were eaten.

Since there are many people who habitually consume considerable amounts of foods that have been found to possess activity and who do not develop goiters, a marked variation in the response to goitrogenic stimuli probably exists. Clinical experience has shown this to be the case in the requirements for antithyroid medication in thyrotoxicosis.

Probably there is a good deal of variation in the potency of foods at different times also. Webster (1932) reported that the goitrogenic activity of cabbage obtained from the same source became much more marked with the approach of the winter months.

The antithyroid material consumed by the vast majority of people eating such foods would probably not be sufficient to have any visible effect on the thyroid. Only if the major portion of one's dietary intake were limited to goitrogens would thyroid hyperplasia be likely to result. However, under conditions of iodine deficiency, the added stimulus of goitrogenic food might produce hyperplasia in a thyroid gland which was just able to produce sufficient hormone to satisfy the metabolic requirements of the body, while still retaining its normal size.

The nature of the active antithyroid compounds in these foodstuffs is unknown. Active material could be extracted with ether from the clear aqueous extract of rutabaga. When the resulting ether extract was dried, the active material would dissolve in a small volume of water. Such a purified extract, equivalent to 2617 gm. rutabaga, completely inhibitied the uptake of iodine for a full twenty-four hours when ingested.

SUMMARY

The antithyroid effect of sixty-one different foods was tested in one hundred different subjects by using radioactive iodine. Of these, rutabaga was found to possess the greatest antithyroid activity. Vegetables were more active than animal products and the most potent vegetable families were the Chenopodiaceae, Compositae, Cruciferae, Cupuliferae, Juglandaceae, Leguminosae, Rosaceae, and Umbelliferae. Cow's milk, beef liver, and oysters also showed some activity.

The active principle of rutabagas could be extracted from a watery solution with ether and was contained in the water-soluble portion of this dried ether extract.

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NOTES AND COMMENTS

HYPERVITAMINOSIS A AND THE DISTRIBUTION OF BODY IODINE

THE RESULTS of numerous investigations on vitamin A and thyroid physiology (Drill, '43; Simkins, '47) have uncovered relationships which require further investigation. Experiments dealing with the effects of thyroxine administration, or of thyroidectomy on vitamin A storage will not be considered in this paper; these studies have been adequately reviewed in the publications cited above. The effects of vitamin A-deficiency and, especially, of hypervitaminosis A on thyroid function are of primary interest to the present discussion. Briefly, avitaminosis A produces hypertrophy of the thyroid in female and atrophy of the thyroid in male rats (Coplan and Sampson, '35), and an increase in thyroid weight (Coplan and Sampson, '35; Lipsett and Winzler, '47); some of the thyroid follicles exhibit the changes of colloid goiter (Lipsett and Winzler, '47). Conversely, large doses of vitamin A reduce thyroid weight (Sadhu and Brody, '47) and reduce the colloidal material in the thyroid follicles (Sherwood, et al, '34).

The histological changes mentioned are accompanied by marked effects on iodine storage and utilization. The inability of the thyroid to regulate iodine normally, in the present of A-deficiency, has been amply demonstrated (McCarrison, '30; Mitzkewitsch, '34; Coplan and Sampson, '35; Lipsett and Winzler, '47). A relationship between iodine metabolism and vitamin A has also been indicated by studies utilizing large oral doses of the vitamin (Lipsett and Winzler, '47; Simkins, '47; Truscott and Sadhu, '48a).

It is conceivable that the effects of vitamin A on the thyroid and on iodine metabolism are inseparable factors and are mediated by a pituitary-thyroid relationship. This is suggested by the work of several investigators (Elmer, et al, '35; Fellinger and Hochstadt, '36; Schulze and Hundhausen, '39; Truscott and Sadhu, '48a). Such studies have indicated, directly or indirectly, that hypervitaminosis A exerts an inhibitory action on the secretion of the thyrotrophic hormone of the pituitary. The following experiments were undertaken to gain some insight into the mechanism by which vitamin A might exert this action.

MATERIALS AND METHODS

The animals used in this investigation were adult male albino rats, which were divided into one control and two experimental groups. The animals in the first experimental series received a daily oral dose, per rat, of 15,000 I.U. of vitamin A administered as Afaxin.[1] The second experimental group received a daily oral dose of 20,000 I.U. per rat, given as percomorph oil. The length of the dosage periods were 14 days for the first and 18 days for the second group.

Received for publication June 1, 1948.

[1] Vitamin A concentrate furnished by the Winthrop Chemical Co.

The rats were sacrificed at the end of the experiment, and the protein-bound iodine of the tissues was determined by the method of Salter and McKay ('44).[2] Vitamin A values were obtained by the antimony trichloride method, with the use of a Lumetron photoelectric colorimeter.

RESULTS

Hepatic iodine was markedly decreased in the hypervitaminotic animals (table 1), especially in those receiving the smaller dose of the vitamin; the magnitude of the iodine decrease, however, was not proportional to the amount of vitamin A administered.

The low level of hepatic iodine made it advisable to determine whether this formed part of a general depression of iodine content in other tissues of the body. Because the iodine content of the pituitary, and particularly of skeletal muscle is normally high, it was believed that any general alteration in iodine distribution would be reflected in the iodine content of these tissues. In contrast to the decrease in hepatic iodine, both pituitary and muscle iodine was significantly increased. It appeared unlikely, therefore, that the iodine level in the liver was due to a general lowering in the iodine content of body tissues.

Protein-bound serum iodine was increased and was directly related to the dosage level of vitamin administered. There was an increase of 17.1% and 34%, respectively, in the Afaxin- and percomorph oil-fed animals; a statistically significant change however, was present only in the percomorphed rats. In agreement with the results obtained in liver analyses, the magnitude of the changes in serum iodine was not proportional to the dosage level of the vitamin.

TABLE 1

Body weight (gm.)	322	185	292
Number of animals	20	12	18
Thyroid weight (mg. %)	6.18	5.03	4.03
Serum iodine (γ/100 ml.)	3.21 ±0.12	3.76 ±0.92	4.33 ±0.76*
Thyroid iodine (γ/100 mg.)	90.4 ±3.3	82.1 ±4.5†	67.3 ±4.8*
Hepatic iodine (γ/gm.)	0.201 ±0.021	0.097 ±0.013*	0.120 ±0.05*
Muscle iodine (γ/gm.)	0.2243 ±0.586	—	0.3443 ±0.213*
Pituitary iodine (γ/100 mg.)	0.130±0.009	0.253 ±0.015*	0.226 ±0.017*
Serum vitamin A (I.U. %)	85.0	260	420
Hepatic vitamin A (I.U. %)	408.0	7188.5	11009.5

* $p < .01$.
† $p < .05$.

These results suggested that there was a decreased destruction of thyroxine by the liver, thus leading to an increase in circulating thyroxine. It would be expected, therefore, that thyrotrophic hormone secretion would be depressed with an eventual decrease in both weight and iodine content of the thyroid gland. Consequently it was of interest to observe that, in addition to the increase in pituitary iodine, there was a reduction in thyroid weight and iodine content; furthermore, these changes were related to the amount of vitamin administered, the lower values existing in the group receiving the larger amount of the vitamin.

[2] The authors wish to thank Dr. Wm. T. Salter for placing laboratory facilities at their disposal for the iodine determinations.

Ineffective storage and distribution of the administered vitamin in the experimental groups remained a possibility. The liver and blood levels of vitamin A were determined in an attempt to ascertain whether the above possibility could be ruled out. It will be noted (table 1) that the high values obtained fall within the expected range and are directly related to the amount of vitamin fed.

DISCUSSION

The reduction in thyroid iodine and thyroid weight, following oral administration of large amounts of vitamin A, supports the concept that the vitamin may exert an inhibitory action on thyrotrophic hormone secretion (Elmer, et al, '35; Fellinger and Hochstadt, '36; Schulze and Hundhausen, '39; Truscott and Sadhu, '48a). Certain investigators (Belasco and Murlin, '40; Sadhu and Brody, '47) have suggested that the iodine in the thyroxine molecule is picked up by the double bond of the vitamin; the vitamin A-iodide thus formed may depress the secretion of thyrotrophic hormone and result in a decrease in thyroid size. Sadhu ('48), however, observed that this action was more marked when vitamin A was fed alone than when it was administered in conjunction with iodine in the diet. He concluded, therefore, that the vitamin does not reduce thyrotrophic hormone secretion by the formation of a vitamin A-iodide or -thyroxine like compounds.

A possible explanation of the above results is suggested by the data presented in this paper. Following oral administration of large doses of vitamin A, there appears to be a lessened destruction of thyroxine by the liver; it has been demonstrated (Truscott and Sadhu, '48b) that this is not due to a displacement of thyroxine iodine from hepatic tissue by the accumulation of excessive amounts of the vitamin in the reticuloendothelial cells. The consequent hyperthyroxinemia affects the pituitary depressing the secretion of thyrotrophic hormone; this is indicated by the high values of serum and pituitary protein-bound iodine obtained in these animals. With depression of thyrotrophic hormone secretion, there is the observed decrease in protein-bound iodine and in weight of the thyroid gland.

SUMMARY

The distribution of body iodine was studied in albino rats receiving large oral doses of vitamin A.

Hypervitaminosis A was accompanied by a decrease in protein-bound iodine in liver and thyroid, and by an increase in protein-bound iodine in serum, pituitary and skeletal muscle.

It is suggested that these results are due to a decreased hepatic destruction of thyroxine, with a consequent hyperthyroxinemia; the latter depresses thyrotrophic hormone secretion, producing the observed decrease in both thyroid weight and in protein-bound iodine in the thyroid.

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RENAL FUNCTION IN THE ADRENALECTOMIZED RAT[1]

DESPITE the voluminous literature concerning the effects of adrenalectomy in a wide variety of species, surprisingly few attempts have been made to assess the immediate effects on renal function. Talbott et al (1942) studied renal function in patients with Addison's disease and in patients with adrenal insufficiency seconday to pituitary pan hypofunction. They reported that while the ordinary clinical tests showed no aberration, more precise quantitative procedures, using inulin and diodrast, showed impairment of all measured functions. This impairment included the glomerular filtration rate, renal plasma flow and tubular excretory as well as reabsorptive mass. More recently, Sanderson (1948) has studied renal function in four cases of untreated Addison's disease not in crisis, and noted a similar depression of all functions.

White, Heinbecker and Rolf (1947) studied the inulin and diodrast clearance in two adrenalectomized dogs. They observed that mild adrenal insufficiency, in which the blood volume, NPN, and sodium and potassium levels were all normal, was accompanied by a great decrease in C_D and Tm_D with a lesser fall in C_{IN}. These changes were more completely restored by the implantation of desoxycorticosterone acetate pellets than by adrenal cortical extract. The effect of saline maintenance alone was not studied.

In view of the azotemia which may be observed in patients with Addison's disease, it seems important to ascertain more definitely just how far the kidney may play a contributory rôle. Experiments on rats were undertaken to determine the immediate effects of adrenalectomy with saline maintenance on renal function in this animal.

EXPERIMENTAL

Three separate experiments were carried out to assess renal function 1, 4, and 6 days after bilateral adrenalectomy. For each experiment, mature hooded male rats, weighing approximately 300 grams were selected. For the first two experiments, the animals were slowly adapted to tube feeding and were maintained at constant intake for at least one week prior to operation.

Received for publication June 14, 1948.
[1] This work was supported by a grant from the Life Insurance Medical Research Fund.

The animals of the third experiment received Purina Fox Chow ad lib. Adrenalectomy was performed through bilateral lumbar incisions and following this, both control and adrenalectomized animals were maintained with 1% saline as drinking water while tube feeding was continued. For the first experiment, the control animals were subjected to a sham adrenalectomy. As this procedure was without apparent effect on renal function as determined one day later, it was not used in subsequent control groups. Renal function was determined using inulin and sodium p-aminohippurate by the method previously described for the comparative investigation of groups of rats (Friedman, Polley and Friedman, 1947). Control and test animals were studied at the same time.

TABLE 1

Time post adrenalectomy	Expt. 1		Expt. 2		Expt. 3	
	1 day		4 days		6 days	
	Sham operated	Adrenal-ectomized	Untreated control	Adrenal-ectomized	Untreated control	Adrenal-ectomized
C_{IN} cc./100 cm.²/min.	0.24 ±0.03	0.30 ±0.03	0.23 ±0.03	0.23 ±0.05	0.27 ±0.04	0.22 ±0.04
C_{PAH} cc./100 cm.²/min.	2.14 ±0.31	1.46 ±0.34	2.13 ±0.33	1.59 ±0.22	2.12 ±0.31	1.92 ±0.27
Tm_{PAH} mgm./100 cm.²/min.	0.107 ±0.006	0.067 ±0.012	0.108 ±0.010	0.088 ±0.021	0.114 ±0.010	0.110 ±0.015
FF as %	11.3 ±2.5	21.8 ±6.1	11.2 ±1.6	15.4 ±6.0	12.6 ±1.4	11.7 ±2.2
C_{PAH}/Tm_{PAH}	19.8 ±2.1	22.2 ±6.8	19.7 ±2.7	18.2 ±1.6	18.7 ±2.7	17.4 ±1.0
No. of animals	4	4	7	7	7	7
Average body weight	307	318	330	318	290	284

Table 1 presents the findings. It is apparent that kidney function as determined in the three control groups, made up of different animals studied at entirely different periods, did not vary, and hence represents a good standard of comparison for the test animals. The spread of data is here rather less than usually observed with this method, probably due to the standardized feeding as well as to the large size of the animals. It is also noteworthy that the normal values shown here, especially that of C_{IN}, are lower than those observed in albino rats of the Wistar strain which we have previously reported. The data indicate that this is in part referable to a strain difference, and in part to the larger size of the animals used here.

One day after adrenalectomy a marked fall in renal plasma flow, C_{PAH}, was observed, together with a parallel decrease in the functioning tubular excretory mass, Tm_{PAH}. This was not accompanied by any decrease in the glomerular filtration rate, C_{IN}, which, in fact, was slightly elevated above the control value.

Four days after adrenalectomy renal function was still depressed, although to a lesser extent. Again, C_{PAH} and Tm_{PAH} were the functions most affected, while C_{IN} remained unchanged.

Six days after adrenalectomy renal function was essentially normal.

Repeat experiments were performed on groups of Wistar albino rats. The same initial depression followed adrenalectomy, and when the animals were

maintained with 1% saline as drinking water renal function was restored to normal at 6 days.

DISCUSSION

It is evident that immediately after adrenalectomy renal function is seriously impaired in the rat. The pattern of derangement is essentially that observed in the dog by White, Heinbecker and Rolf (1947) except that in their experiments C_{IN} was reduced, although to a lesser degree than the other functions studied. This need not represent an absolute difference between the species since the modifications in procedure necessary for estimating renal function in the rat impose limitations on the validity of such an inter-species comparison.

If the animal survives adrenalectomy and is maintained on saline only, renal function becomes essentially normal six days after operation. The fourth day after adrenalectomy appears to represent an intermediate period, since, as indicated by the spread of the data, some animals are approaching normality more rapidly than others.

The results obtained in the control animals indicate the necessity of mentioning the strain of rat employed in reporting clearance values in this species.

SUMMARY

One day after adrenalectomy in the rat (hooded and Wistar albino) there is a marked fall in C_{PAH} and Tm_{PAH}, while C_{IN} remains unaffected, as revealed by clearance studies using inulin and sodium p-aminohippurate.

Renal function in the adrenalectomized rat maintained with saline becomes essentially normal six days after operation, an intermediate phase in the restorative process occurring at four days.

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ACKNOWLEDGMENT

The sodium p-aminohippurate used in these experiments was supplied through the courtesy of Dr. W. P. Boger, Sharp and Dohme Inc.

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ERRATA

In the paper by Kleitman and Ramsaroop, entitled "Periodicity in Body Temperature and Heart Rate" which appeared in ENDOCRINOLOGY 43: 1-20 (July), 1948, the following errors have been noted:

On page 8, line 12 from bottom, "fig. 3" should read "fig. 5."

On page 8, lines 8 and 9 from bottom, "1.19° (97.34°-98.53°)" should be interchanged with "1.20° (97.93°-99.13°)" on line 7 from bottom.

ERRATA

In the paper on Nitrogen and Ramsayite entitled "On the Rocks in Rudh' Leacanach and Their Relations to Igneous Intrusions," which appeared in June, 1930, the following errors have been noted:

On page 8, line 12 from bottom, "fig. 3" should read "fig. 4."

On page 8, lines 8 and 9 from bottom, "(120° (67°30′ : 90° (22°30′)" should be exchanged with "(120° (67°30′ : 90° 15°)" on line 7 from bottom.